

**INVESTIGATION OF PRACTICAL HURDLE TECHNOLOGIES  
FOR PREVENTING PHOTOOXIDATION OF CURED LUNCH MEATS  
IN PREPACKAGED SANDWICHES.**

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## Abstract

Discoloration of cured meats in the retail case is a concern for prepackaged sandwich manufacturers, resulting in negative consumer perceptions of quality and freshness, and decreased purchase intent. The introduction of Modified Atmosphere Packaging (MAP) has significantly improved color appearance and increased the refrigerated shelf life, but it has not eliminated cured meat discoloration from developing. Consumer demand to see the product has led to greater product exposure to light, resulting in cured meat discoloration via photooxidation.

The mechanism of photooxidation of cured meats is not fully understood, and there are multiple factors to consider including package variables (residual oxygen, Oxygen Transmission Rates (OTR), and product to headspace ratio), storage and display conditions (frozen followed by refrigeration, light exposure), and challenges created by the combination of bread, meat and cheese for a 30 day refrigerated shelf life in order to find practical, cost effective solutions for the prepackaged sandwich industry that are acceptable to the consumer.

Using a bestselling ham & cheese sandwich variety in conjuncture with a 80% N<sub>2</sub>/20% CO<sub>2</sub> Modified Atmosphere Package, a number of potential solutions are evaluated including ferrous and nonferrous based oxygen scavengers, UV (ultraviolet) blocking films, ham formulations and LED vs. fluorescent lighting to understand the impact to cured meat color scores in the form of  $L^*$  and  $a^*$  color measurements, visual appearance, and residual oxygen over time. Solutions demonstrating visual improvements or better color stability on  $L^*$  or  $a^*$  color values are presented to consumers for preference and input.

The results of the study show that Ferrous based oxygen scavengers have potential, but challenges are created when used in a frozen storage and distribution system followed by refrigerated display. While predicted  $a^*$  values in a scavenger/MAP packaged ham are higher (more red) over time compared to MAP only, the high variability found in initial meat color, compounded by variability in package oxygen content and changing conditions in the package over time results in no statistical differences due to predicted overlapping  $a^*$  rate constants. The impact to  $L^*$  (lightness and darkness) values are measurable and correlate with observed consumer preferences, but inconsistent

throughout the refrigerated shelf life, with the potential for scavenger sachet packaged ham sandwiches to develop low  $L^*$  (darkening) values that are unacceptable to the consumer. As a result, consumer preference for sandwiches with and without an O<sub>2</sub> scavenger is inconsistent at different days throughout the shelf life. Consumer input on active packaging reveals the need for further understanding of acceptance in a retail environment where the demand for fresher products continues to grow. While unaided, the sachet is initially unnoticed by consumers implying acceptance, awareness of its presence leads to concerns over freshness. The added cost of a scavenger requires recovering lost sales of 80,000 sandwiches per year, but brand perception also needs to be considered. Grey tinted ferrous based scavenging film was not accepted and viewed as an attempt to hide qualities of the food.

Consumer demand for higher quality ham with full muscle appearance and texture along with the many unknown factors in cured meat pigment formation and photooxidation mechanism makes ham reformulation not a practical approach.

Use of LED lights and UV blocking films in the UV range of 366 – 400 nm that is detrimental to meat pigments did not result in improved color scores or visual appearance, validating that visible light is as equally destructive to meat color.

As a result of this study, no changes are recommended to the current MAP package in a frozen storage and distribution with direction for future studies provided.



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# **1. Introduction**

## **1.1 The sandwich market**

The definition of a sandwich according to Wikipedia is “a food item consisting of one or more types of food placed on or between slices of bread, or more generally any dish wherein two or more pieces of bread serve as a container or wrapper for some other food” (Wikipedia, 2014). The history of sandwiches dates back to the 1st Century B.C. and has continued to evolve into a significant food format, particularly in Western cultures (Stradley, 2014).

In today’s market, there are many formats for sandwich building including preparing at home, restaurants, Quick Serve restaurants & individually wrapped prepackaged “grab & go” options. For pre-packaged sandwiches, there are multiple locations within a store to purchase from (including refrigerated display, hot display applications, and frozen) but “grab & go” for the purpose of this evaluation is further defined as pre-packaged sandwiches that are ready to eat foods for immediate consumption and available through the refrigerated case.

The landscape for “grab & go” retail locations has continued to evolve, with convenience stores and grocery being a niche, but a major growing option in the industry. According to the International Dairy, Deli, Bakery Association (IDDBA) whose mission is to promote the growth and development of dairy, deli, and bakery sales in the food industry, 47% of sandwich purchases are made at a fast food outlet and sub shops, 12% at Restaurant, 5% at Supermarket, and 2% at Convenience store (IDDBA, 2014). Though a small overall percentage of the total market, the dollar sales for the Supermarket category are significant. Sandwiches represent 10.7% of the prepared food option sales, totaling \$1.9 billion dollars annually (IDDBA, 2015). For convenience stores, the prepackaged sandwich industry in the US is a \$500 million dollar industry with 179 million prepackaged sandwiches purchased annually. (Mintel, 2014) Sandwich sales within convenience stores continue to grow, with 7.2% growth reported in 2011 (Longo, 2012). E.A. Sween company (does business as Deli Express®, also recognized as EAS) is a leader in the pre-packaged sandwich industry. Founded in 1955, Deli Express continues to thrive today with an SQF level 2 manufacturing facility producing approximately 1 million sandwiches per week. The privately held company employs 900 people across

the country and distributes to 26 states servicing over 15,000 stores. EAS operates a primarily frozen distribution system with limited refrigerated distribution (Deli Express, 2014).

Deli Express® is the number one selling convenience store sandwich, with Ham & cheese wedge the top selling sandwich variety (Table 1.1). Three out of the top ten sandwiches purchased have ham (Mintel, 2014).

**Table 1.1** Mintel reported sandwich sales for 52 weeks ending Feb. 23, 2014.

TOTAL U.S. - Convenience AllScan	Latest 52 Weeks Ending Feb 23, 2014	
Report has been sorted based on Dollar Sales of refrigerated sandwiches - excluding Private Label	Brand	Dollar Sales
DELI EXPRESS RFG SMOKED HAM & CHEESE SANDWICH WEDGE 4.6OZ 4143302122	DELI EXPRESS	\$9,402,915
DELI EXPRESS RFG SMKD TURKEY & CHEESE SANDWICH 5.8OZ 4143302126	DELI EXPRESS	\$9,244,904
DELI EXPRESS RFG CHICKEN SALAD SANDWICH WEDGE 5OZ 4143302118	DELI EXPRESS	\$9,173,737
DELI EXPRESS RFG OVEN RSTD TRKY & CHS SANDWICH WEDGE 4.2OZ 4143302119	DELI EXPRESS	\$8,984,129
LANDSHIRE RFG HAM AND CHEESE SANDWICH WEDGE 4.5OZ 9748800040	LANDSHIRE	\$8,961,791
JIMMY DEAN RFG CROISSANT BREAKFAST SANDWICH 4.9OZ 7790011859	JIMMY DEAN	\$7,848,997
DELI EXPRESS RFG CHARBROIL & CHEESE SANDWICH 9.6OZ 4143302362	DELI EXPRESS	\$7,603,843
LANDSHIRE RFG SMKD TURKEY & CHEESE SANDWICH WEDGE 4.5OZ 9748800043	LANDSHIRE	\$6,939,634
DELI EXPRESS RFG SMOKED HAM & CHEESE SANDWICH SANDWICH 6OZ 4143302127	DELI EXPRESS	\$6,658,172
LANDSHIRE RFG CHICKEN SALAD SANDWICH WEDGE 4.5OZ 9748800048	LANDSHIRE	\$5,909,013

Prepackage sandwich sales continues to be driven by “on the go” Americans, with 46% of sandwich purchases happening away from home (Technomic, 2014). Today, consumers eat an average of 3.6 sandwiches per week, with three out of every five sandwiches (61%) taken to go (Technomic, 2014).

## 1.2 The Consumer and needs

Who the consumer is, and their expectations is complex, but does have some central themes which include a desire for fresh, high quality foods, while being a value (IDDBA, 2014; Technomic, 2014). In some venues such as grocery, the expectations are clearer, and the bar set higher. In other venues such as convenience stores or “gas stations”, the willingness to compromise ideals such as fresh & high quality for convenience and filling a “quick need” is more apparent. Also, because many grocery & convenience stores have branded Quick Serve Restaurants (QSR) and food services options within (such as in-store deli cases), who the consumer is and what their concerns & expectations are across all venues are important to distinguish who the true consumer is. When a consumer is asked if they bought a pre-packaged sandwich from a grocery or

convenience store, some may not distinguish between a pre-made sandwich versus one made for them in the store. Both are ultimately served in a wrapper.

In an online survey of 1000 grab & go convenience store sandwich users commissioned by Deli Express® (DE new sandwich feasibility), male consumers were more likely to purchase a pre-packaged sandwich on a weekly bases than women (23% vs. 13%), and are under 54 years of age, and want higher quality sandwiches, but feel convenience stores face quality and consumer satisfaction hurdles. The respondents reported that the primary reason for selecting pre-packaged grab and go sandwiches are freshness, convenience, speed, taste, and price.

In a similar study focused on grocery consumers (DE new sandwich feasibility – Grocery stores), men were also more likely than women to purchase a grab & go sandwich on a weekly basis (20% vs. 16%), the average age was more likely to be 37 or younger, and also expressed a concern over the industry being able to deliver on quality and consumer satisfaction. A key insight for this study was that while craving & convenience are primary drivers, consumers still maintained high expectations by looking for visual cues that signal freshness and quality.

According to the International Dairy, Deli Bakery association, value trumps all considerations, but freshness is a differentiator. Survey data collected in 2013 showed 52 % of Grocery Deli shoppers rated freshness as extremely important, but only 21% felt the criteria was being met (IDDBA, 2013). Deli store shoppers also ranked “food items that are visually appealing” as the number two influential factor when shopping a Deli (behind overall clean & sanitary area (IDDBA, 2013). The demographic profile of the Grocery Deli & Specialty meat purchaser is 41% Male, 59% female; 80% Caucasian, 13% African American, and 7% Other; 40% 55+ in age, followed by 33% Millennial (18-35), and 23% Gen X (36-49) (IDDBA 2014).

Similarly, Technomic evaluated restaurant chains for sandwich desires and found that quality, taste, value & price are the most important considerations when purchasing a sandwich (Technomic, 2014). The demographics of this group of consumers was 51% female, 49% male; 65% Caucasian, 16% Hispanic, 13% black, 4% Asian; and is split somewhat equally amongst age groups, with the majority 55 + (33%). Other Key findings regarding sandwich consumption behavior also included: Grocery stores

purchases for sandwiches are on par with local independent delis and cafes, half of total consumers and two thirds of the younger consumer segment say they purchase grab and go sandwiches, lunch is more important for portability than dinner, and quantity and appetizing appearance continues to increase (Technomic, 2014).

### 1.3 Color expectations

The consumer's first impression of food is often the color & appearance, particularly in meat, fruit & vegetables (Lawless and Heymann, 1999). Consistently, consumer preference data exists to support that color is a significant factor influencing consumer purchase intent. In a study published in the *Journal of Food Quality*, color was identified as one of the most important sensory attributes of a food, affecting consumer judgment of other sensory characteristics (Clydesdale, 1991). Because for many foods color is associated with changes in aroma, taste, & flavor; people are conditioned to see any color change as affecting all aspects of the product. In the case of meats both cured & fresh, the color change is often attributed to a food safety concern, though eating satisfaction has proved unaffected (Carpenter, Cornforth, and Whittier, 2001). In examining steaks, consumer preference for beef color influenced the likelihood of purchase, but did not bias eating satisfaction (Carpenter, Cornforth, and Whittier, 2001). Fresh meat purchasing decisions are strongly influence by color more than any other factor because poor color is an indication of product that is not fresh or wholesome (Mancini & Hunt 2005). 15% of retail fresh beef is discounted because of discoloration (Smith et al., 2000). Color stability of Modified Atmosphere packed cured cooked ham is one of the most important characteristics as it is the primary quality attribute seen by the consumer (Nannerup et al., 2004).

The expectation of cured meats being a "typical color" is so important, that it becomes the gold standard by which alternatives are compared to. In an investigation of how uncured hams were accepted by consumers (compared to the cured alternative), Higher  $a^*$  value scores (indicating redness) corresponded to a higher consumer acceptance (Table 1.2). A cured ham with an  $a^*$  of 20.5 received a consumer acceptance score on 6.68 (out of 9), while an uncured ham with an  $a^*$  value of 16.6 received a score of 5.29.



The higher consumer scores correlated to the ham with the highest  $a^*$  value (Sindelar et al., 2007).

**Table 1.2** ham consumer acceptance scores correlated with  $a^*$  score.

type	$a^*$ score	consumer acceptance score
Brand A (uncured)	18.9	6.29
Brand B (uncured)	16.6	5.29
Brand C (uncured)	22.0	6.96
Brand D (uncured)	17.6	5.16
Brand E (cured)	20.5	6.68

The color of ham is a function of two factors: the meat pigment (myoglobin content), and light scattering properties (Varnam and Sutherland, 1995). For sandwich manufacturers like E.A. Sween Company, the challenges of the future are to deliver food for people on the go that meet consumers' expectations of being perceived as fresh & healthy at a price point that is deemed as a good value.

## 1.4 Ingredients

The primary components for a sandwich are the meat, bread & cheese. The leading sandwich proteins for consumption according to Technomic are Chicken (27.1%), Turkey (15.1%), Ham (14.2%), Bacon (12.2%) & Roast Beef (5.5%) (Technomic, 2014). There is increasing importance on the quality of bread, cheese and condiments today vs. 2012, but high quality meat expectations has long been deemed important and that importance remains unchanged in 2014 (Technomic, 2014).

Within the category of ham, there are many terms to become familiar with as they identify the composition and character of the end product. For example, a chopped ham may have up to 15 percent shank meat, which is 3% more than a normal whole ham (NAMP 6<sup>th</sup> edition, 2010). This is relevant as differences in the myoglobin content can occur between animals of the same species and between muscles from the same animal (Varnam and Sutherland, 1995). The terms "Ham," "Ham with Natural Juices," "Ham, Water Added," "Ham and Water Product" all indicate how much water remains in the

ham after its final processing (NAMP 6<sup>th</sup> edition, 2010). Because added water dilutes the natural protein content, it is relevant to the end color intensity of the product. Deli Express<sup>®</sup> uses water added smoked ham in its bestselling Ham & cheese sandwich. Table 1.3 lists the amount of added water allowed for several categories of ham.

**Table 1.3** Categorization of ham with % protein content and amount of added water allowed

Product description	protein in %	Amount of allowed added water
Ham (Dry Cured)	20.5	0
Ham with Natural Juices	18.5	less than 8
Ham - Water added (Deli Express ham)	17	12 to 15
Ham & Water products	15	more than 15
Restructured ham	Multiple muscles used, fat levels vary (typically higher fat)	

Although not in a traditional sense, Carbon Dioxide, Nitrogen, and the Modified Atmosphere Packaging (MAP) are also ingredients in the final on shelf product. Consumers use meat color as an indicator of wholesomeness, and MAP with the right blend of gases can maximize initial color and as well as color stability in meats (Mancini & Hunt 2005). Without modified atmosphere packaging, the pink color that defines ham color expectation of the product deteriorates rapidly.

## 1.5 Statement of the problem

Cured meat in a sandwich can discolor rapidly in a gas flushed package with low Oxygen as the result of the cured meat pigment nitrosylmyoglobin (pink) being oxidized to metmyoglobin (brown) in the presence of light (Møller et al., 1999). In the case of thermally induced auto oxidation, the stoichiometry of pigment and oxygen is 1 to 1, but in photo-oxidation, this ratio shifts to 1 to >1, resulting in more discolored pigment at low residual oxygen (Møller, Weber and Bertelsen, 1999). Several conditions accelerate this issue including light sources present, product to headspace volume ratio, Oxygen transmission rates (OTR) of the film, and the temperature of the cooler (Nannerup et al. 2004). This issue affects both Deli Express<sup>®</sup> customers (defined as business's that merchandise the product) and consumers who ultimately consume the sandwich. This

phenomenon does not consistently occur and is not predictable, but has resulted in lost business potential, existing customer's dissatisfaction, consumer complaints, and Deli Express employee's constantly monitoring and purchasing sandwiches off the shelf to remove a product that could be taken as spoiled. There is some customer & consumer awareness of the problem and potential solutions, but not a complete understanding of the scope of the issue, solutions available, or consumer opinions on the subject.

One customer reported *"I know we've talked about this before but our bad "merch" on the Mega Wedge ham sandwich is double that of the turkey and I'm sure it's largely driven by the discoloration of the ham over time. I realize that this has to do with the lighting but the fact of the matter is that this situation needs to be fixed. One manufacturer said they put an additional layer of some special type of film to prevent this from happening to their ham. Is that something you can do? If not, we may need to consider other manufacturers. The bad "merch" is hurting our profitability on this item."* (E. Kouri, Kum and Go, personal communication, September 09, 2014). Another potential customer reported that simply putting a sandwich into the open air cooler with the light on over the weekend resulted in a discolored meat.

Covering up the cured meat with a large label or opaque packaging eliminates the issue, but consumer demand is to see what they are purchasing.

In 2013, Deli Express received two hundred and ten product complaints out of seventy million produced sandwiches. While consumer complaints regarding meat discoloration are few (0.00027%), comments made indicate consumer recognition of the issue.

Deli Express consumer comment log:

- Smoked Ham & Cheese on White (4/9/2013): Thought the ham was turkey as the top side was white; the bottom of the meat was colored like ham. Took 1 bite and it tasted horrible.
- Mega Smoked Ham & Cheese (06/27/13): Color is bad/off.
- Mega Italian Sandwich (7/15/2013): Customer said the meat on the Italian Wedge she purchased was discolored. There was no date on the package. Did not feel comfortable eating it.
- Mega Smoked Ham & Cheese (7/22/2013): the Ham was white and looked more like turkey than ham.

## 1.6 Economics

There is a financial impact to be considered. According to survey conducted by Technomic (Deli Express New Sandwich feasibility survey), while consumer reasons for purchasing pre-packaged sandwiches includes “fresh”, it also includes price. Lost sales can be measured in the form of “buy back” or shrink (expired product at point of sale) programs. Deli Express’s route system has a guaranteed sales program that can be measured. This does not represent all sales company wide, but is directional to the potential financial impact of the issue.

Product that has to be removed from the shelf (shrink) has multiple causes including expired shelf life, “leaker” packages (gas flushed packages with an incomplete seal), and discolored sandwiches. Categorization of causes for shrink isn’t tracked, however If meat discoloration was responsible for even 10% of unit “shrink”, elimination of buy back on the two top selling Ham sandwiches would result in an annualize potential savings of \$33,800 (Table 1.4).

**Table 1.4** Measured lost sales as the result of shrink on two varieties of ham sandwich.

Product description	Annual gross unit sales	Shrink units	Value of shrink units
Ham and Cheese wedge single	3,300,000	98,000	\$180,000
Ham and Cheese wedge Mega sized	1,700,000	68,000	\$158,000
<i>totals</i>	5,000,000	166,000	\$338,000
			\$33,800

Though the pay back of a solution can be justified against this savings, the impact of meat discoloration to future sales and attitudes toward the brand are unknown. Given that the color is often viewed as being not wholesome, lost sales is not the only consideration.

## 1.7 Research needs

While there is research evaluating cured meats and cheese separately, there is very little research evaluating Ready-To-Eat (RTE) sandwiches that contain multiple ingredients of varied properties and the potential complex interactions that may occur between these ingredients. There are many intrinsic and extrinsic factors to consider when investigating

this topic which will be covered in the literature review, but the intent of this research is to investigate if there are practical solutions for the pre-packaged sandwich industry to implement that addresses the meat discoloration issue. This includes a focus on product and packaging solutions, and a better understanding of how storage and light may influence the end color of the cured ham.

## **1.8 Hypothesis & objectives**

My null hypothesis is there are no hurdle technologies available to prevent cured meat discoloration with the variety of commercially used lighting and refrigeration systems for MAP Ready To Eat (RTE) sandwiches that are stored frozen, and displayed refrigerated with a 30 day shelf life.

The research objectives are:

1. Examine key factors in discoloration of meat and establish the best areas of focus.
2. Measure color changes and oxygen content over time to establish performance differences and statistical significance of potential solutions.
3. Gain insight into consumer preference and opinion of the retail product with potential solutions over current state.
4. Evaluation of the financial impact of potential solutions vs. lost sales.
5. Make recommendations for EA Sween Company on options for addressing the issue.

## **2 Literature review**

### **2.1 Historical overview**

The practice of curing meat has been traced back to 1200 BC in Asian countries (Binkerd and Kolari, 1975). The use of desert salts containing nitrates and borax as impurities led to the serendipitous discovery of the “reddening” effect on meats, although written description of this didn’t appear until Roman times (Binkerd and Kolari, 1975). While the primary purpose of curing was preservation of the meats, it evolved to define the appearance and flavor characteristics that define today’s standard of identity (Cassens, 1997). At the end of the 19<sup>th</sup> century, several curing methods were in practice including dry cure, wet cure (also called pickled cure) and combinations of both methods. Nitrite was recognized as the true curing agent, and the source of nitrite from bacterial reduction of nitrate was discovered (Binkerd and Kolari, 1975). The use of nitrite directly in food was approved by the USDA Bureau of Animal Industry in 1925 (Binkerd & Kolari, 1975). Given concerns over the use of nitrites in food and potential links to cancer (Cassens, 1997), the use of nitrite as a curing agent would pose ethical concerns in today’s world (Johnston, Knight, and Ledward, 1992).

From 1900 – 1940 research focused on understanding the chemistry and composition of the pigment colors of cured meat. The role of nitrite interacting with the meat was recognized (and the pigment Nitric Oxide (NO) Haemochromogen identified), but initially it was thought that blood hemoglobin combined with nitrite was responsible for the red and pink appearance of cured meats (Haldane, 1901). In the 1920’s Günther identified that a pigment different from blood hemoglobin was responsible for cured meat color. This pigment in the 1940’s was referred to as nitric oxide myo-hemoglobin and nitric oxide myo-hemochromogen (Urbain and Jensen, 1940). Urbain and Jensen sought to identify the nitric oxide derivatives of hemoglobin in hopes that the findings would apply to nitric oxide myo-hemoglobin, and concluded that oxygen, pH and temperature were major factors in the oxidation (Urbain and Jensen, 1940). The use of modified atmospheres (adding CO<sub>2</sub> to lamb & beef carcasses) to extend shelf life began in Australia and New Zealand in 1930 (Farber and Dodds, 1995).

By the 1950’s, myoglobin was recognized as the principal pigment responsible for cured meat color (Draudt and Deatherage, 1956). It was identified that cured meat prior to

cooking resulted in formation of nitrosomyoglobin and cooked cured meat pigment was referenced as denatured nitrosomyoglobin (Kampschmidt, 1955). Armour and Co. identified that specific light conditions had a more significant role in the discoloration of cured meats. Kampschmidt established that the action of the light was to hasten the dissociation of nitrosomyoglobin into nitric oxide and myoglobin, making the pigment subject to further oxidation (Kampschmidt, 1955). The research also identified wavelengths 400 – 550 nm as the most detrimental to both cooked cured ham (denatured nitrosomyoglobin) and cured ham (nitrosomyoglobin). Using a spectral distribution curve, Kampschmidt demonstrated only slight differences in absorption of the wavelengths of light between the cured meat pigment and cooked cured meat pigment (Kampschmidt, 1955). Draudt and Deatherage identified the major factors in cured meat discoloration to be oxygen, light, and dehydration and that oxygen played a role in both the loss of nitric oxide from the pigment, and the oxidation of the resulting hemichrome (Draudt and Deatherage, 1955). Walsh and Rose concluded that photo-oxidation of cured meat was dependent on light intensity and temperature, but was only mildly impacted by pH (Walsh and Rose, 1956). Hornsey's work on cooked ham attempted to create a method to estimate the stability of the cured meat pigment to light. He concluded that the rate of color fading is exponential, but limited to the light intensity and penetration into the product (Hornsey, 1957).

In the 1960's, work included exploration of adding ingredients to improve stability of the meat pigment in light and the start of Modified Atmosphere Packaging (MAP) solutions with Multivac building their first vacuum chamber machine packaging machine for food packs in 1961 (Multivac). Bailey, Frame and Naumann found that nicotinamide when added with ascorbate to the ham improved color stability from light (Bailey, Frame and Naumann, 1964). The baking industry researched the use of CO<sub>2</sub> gas to retard mold growth, but use of this Modified Atmosphere (MA) wasn't implemented until the 1970's when the German government required food preservatives to be declared on labels (Farber and Dodds, 1995).

In the 1970's, efforts focused on the relationship between the amounts of residual nitrite in cured meat products and the risk of cancer, concluding there was little to no risk to the

public (Cassens, 1997). Mitsubishi introduced the first oxygen scavenger, Ageless<sup>®</sup> (Charles, Sanchez and Gontard, 2006).

The early 1980's saw an increase in the use of Controlled Atmosphere Packaging (CAP) and Modified Atmosphere Packaging (MAP) when the U.K. retail chain Marks & Spenser introduced fresh meats in MAP packaging (Farber and Dodds, 1995). The United States was slow to adopt MAP packaging while Europe readily embraced it, driven in part by the retail centric focus in Europe to reduce waste and sell high quality meats and the shorter geographical distances allowing greater control over the product (Farber and Dodds, 1995). In the late 80's, the US began to use MAP with fresh meat with the retailers Kroger and HEB pioneering MAP packaging and products in the marketplace (Farber and Dodds, 1995). With the new packaging technology came the need to quantify shelf life. Proposed approaches to estimation of shelf life for new products included 1). Literature values for like foods, 2). Distribution turn over for like foods, 3). Distribution abuse test, 4). Gathering consumer complaints, and 5). Accelerated shelf life testing (Labuza, 1982). Awareness of the complexities and confounders involved in the study of food systems that are diverse, complex and actively evolving was created (Labuza, 1982). A chemical kinetic model was developed as a tool for evaluation of complex interactions to provide a better understanding of the causes of food deterioration (Labuza, 1984). Overseas, research continued to evaluate these complexities for cured and fresh meat, including how the animal is handled pre and post slaughter affects end color (MacDougall, 1982) and the effect of packaging conditions and light on ham (Anderson et al., 1988). E.A. Sween Company installed the first MAP machine for the purpose of improving the appearance of cured meat sandwiches and extending shelf life to support a distribution web that had some route sales people only able to return to rural areas once every three weeks.

The 1990's saw growth of MAP in multiple areas including fresh fruit, vegetables and Ready to Eat (RTE) sandwiches. In Europe, MAP was recognized for its role in preventing cured meat discoloration, but also the challenges that retail display of cured meats in low oxygen packaging presented (Anderson et al., 1990). Interactive packaging was explored including oxygen scavengers to slow discoloration (Anderson and Rasmussen, 1992) and other intrinsic and extrinsic factors affecting cured meat



discoloration in modified atmosphere packages were identified (Møller, Weber and Bertelsen, 1999). The use of O<sub>2</sub> scavengers was limited in some countries because of consumer resistance and unclear legislation (Møller et al. 2000).

For the past fourteen years, research has focused on a better understanding of the complex interactions between product formulation, packaging used (including MAP and active packaging), storage shelf life, the mechanism of photo-oxidation, the impact of lighting technologies, and consumer perceptions and acceptance of product. As new and improved techniques have emerged including laser flash photolysis and spin trapping combined with electron paramagnetic resonance spectroscopy, researchers have been able to further explore chemical structure and reaction mechanisms which still are not fully understood (Munk et al., 2010).

## **2.2 Fresh meat chemistry and pigments**

An understanding of the characteristics of raw meat is important as to the extent that the raw meat may have an impact on the color stability of the cured meat version (Møller, Weber and Bertelsen, 1999). The two principle heme pigments (or haem pigments - British English) in muscle are myoglobin and hemoglobin (Hui, 2007). While even a well bled piece of meat can have 20 to 30% hemoglobin present (Fox Jr. 1966), the key heme pigment responsible for color of fresh meat is myoglobin (Varnam and Sutherland, 1995; Johnston, Knight, and Ledward, 1992; Hunt et al., 2012; Fox Jr. 1966). Myoglobin (Mb) is a water soluble globular protein made up of a protein moiety (globin) and a prosthetic group (heme) (Wong, 1989). Myoglobin is responsible for storing oxygen in muscle for metabolic processes (Chang, 1991). The molecular structure contains 8 alpha helices with the prosthetic heme group located in the hydrophobic portion of the molecule. The eight alpha helices are often labeled and referenced as A, B, C, D, E, F, and G (Wong, 1989). The heme group contains a centrally located Iron (Fe) atom (Chang, 1991). The Iron (Fe) atom has six available bonds. Of the six, four connect to the heme ring, the fifth attaches to the proximal histidine-93, and the sixth site is available to bind ligands including oxygen, nitric oxide, carbon monoxide, and water (Hunt et al., 2012). The type of attached ligand and valence of iron dictate the muscle color (Mancini and Hunt 2005). Myoglobin has several redox forms including oxymyoglobin (MbO), Carboxymyoglobin

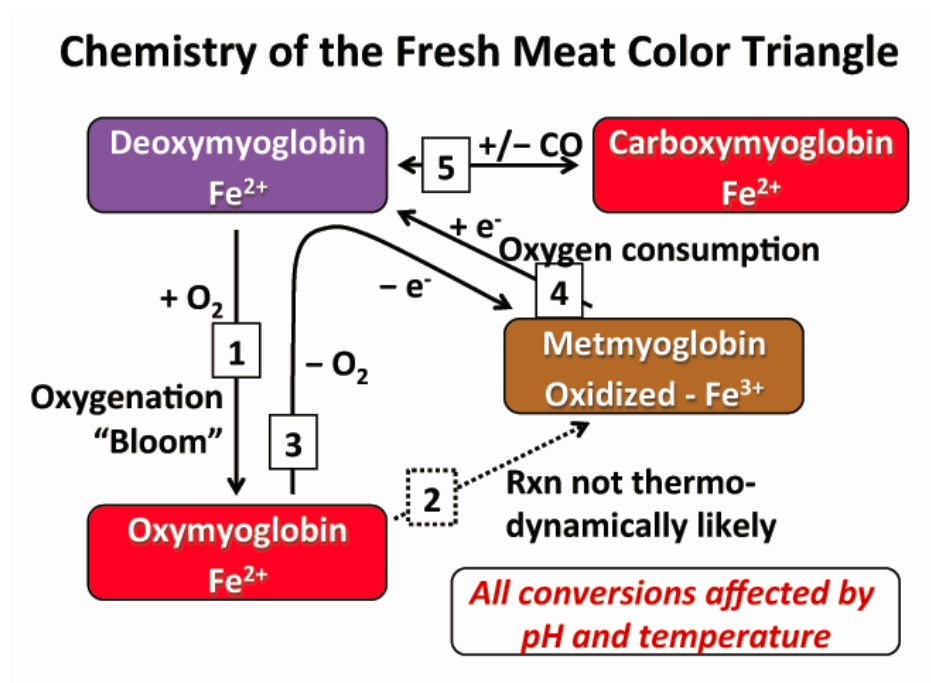
(COMb) and metmyoglobin (MMb) (Figure 2.2) (Hunt et al., 2012 2012; Hui, 2007). The color of fresh and frozen meat is largely determined by the relative Mb, MbO<sub>2</sub>, and MMb present (Bertelsen and Skibsted, 1987). In the presence of oxygen, these three primary pigments exist in a constantly shifting equilibrium based on conditions (Hunt et al., 2012). The competition between myoglobin and cellular mitochondria for oxygen determines the penetration of oxygen on the meat surface, and has a significant influence on the color intensity (Hunt et al., 2012). The interpretation of color is also affected by the light scattering properties of the meat (Varnam and Sutherland, 1995). Temperature and pH history of the muscle post-slaughter also have a significant influence on the color of the final cooked product (Johnston, Knight and Ledward, 1992). The color of the heme pigments are listed in Table 2.1 (Hui, 2007; Hunt et al., 2012).

**Table 2.1** Heme pigments in fresh meat

pigment	Abbreviation	color	ferric state
Myoglobin	Mb	purple	Fe <sup>2+</sup>
Deoxymyoglobin	DMb	purple	Fe <sup>2+</sup>
Oxymyoglobin	Omb	Bright red	Fe <sup>2+</sup>
Metmyoglobin	MMb, MetMb	Brown	Fe <sup>3+</sup>
Carboxymyoglobin	COMb	bright red	Fe <sup>2+</sup>

There are five potential reactions that drive the interconversions of the redox forms of Myoglobin (Figure 2.1) (Hunt et al., 2012). Reaction one depends on time, temperature, pH and oxygen competition. A partial pressure of oxygen over 20.6% favors the oxymyoglobin form (Hunt et al., 2012). Reaction two is unlikely as it is not thermodynamically favored (Hunt et al., 2012). Reaction three is favored under low-oxygen partial pressures of <7 mm Hg (because oxygen is not available to bind DMb, DMb is available to react with hydrogen peroxide to form MMb. The rate is maximal at oxygen pressures of 1 – 1.4 mmHg) (Johnston, Knight, and Ledward, 1992; Hunt et al., 2012). Reaction four is important to meat color stability with oxygen consumption, MMb reducing activity, and postmortem availability of NADH critically influencing the outcome (Hunt et al., 2012). Research suggests that the postmortem pool of NADH can

be regenerated by the addition of malate and lactate, resulting in metmyoglobin reduction (Mohan et al. 2010). Reaction 5 is favored under anaerobic conditions and in the presence of carbon monoxide (Hunt et al., 2012).



**Figure 2.1** Interconversions of myoglobin redox forms in fresh meats. *Courtesy of the American Meat Science Association*

Deoxymyoglobin is subject to further oxidation by oxygen radicals forming metmyoglobin which is characterized by brown pigmentation (Varnam and Sutherland, 1995). To maximize fresh meat color, it is essential to understand the combined effects of two fundamental muscle traits; oxygen consumption and metmyoglobin reduction (Mancini and Hunt, 2005). Metmyoglobin is not present in living tissue as reducing enzymes like NADH will reduce it back to myoglobin (Hui, 2007). The concept for the existence of MMb reducing systems came from observation of the fact that MMb does not accumulate in the muscle color in living animals (Bekhit and Faustman 2005). There are enzymatic and non-enzymatic pathways for reduction of MMb. Enzymatic pathways include NADH-dependent MMb reductase, NADH –cytochrome b<sub>5</sub> reductase, and non-

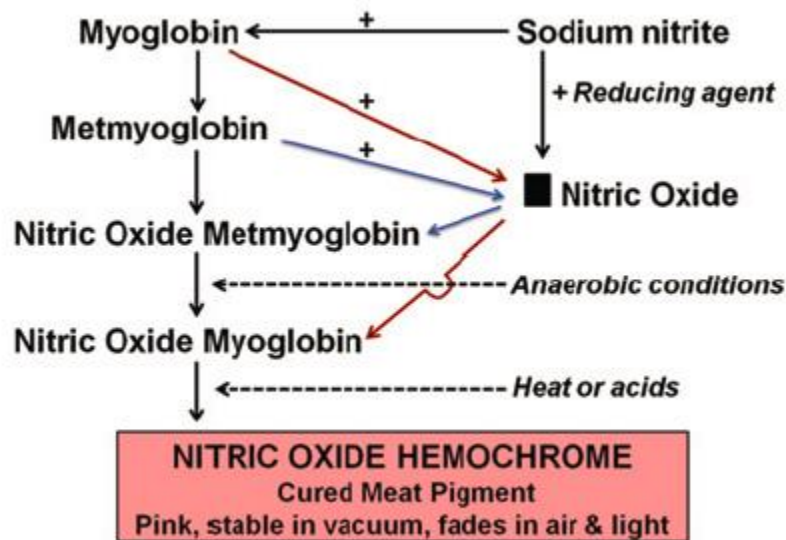
specific reductase (referred to as diaphorase) (Bekhit and Faustman 2005). Proposed non-enzymatic pathways include high concentrations of NADH in the presence of EDTA (Bekhit and Faustman 2005). Manipulation of MMb reducing systems has been a focus in fresh meats to inhibit or eliminate MMb formation and improve fresh meat shelf life (Bekhit and Faustman 2005). A major challenge for all MMb reducing activity is the rapid depletion of the essential cofactor NADH given the normal pH of meat (5.6) (Bekhit and Faustman 2005). This reaction is influenced by several conditions including low oxygen pressure, pH, temperature, time, and enzymatic competition for oxygen (AMSA 2012). Exposure of fresh meat to air causes discoloration of meat generally within 24 hours (Labuza 1982). Fresh meat color is established based on the concentration of hemoproteins present in the meat and the pH and temperature history of the product, and once established cannot be easily changed with further processing (Johnston, Knight, and Ledward, 1992). In gas flushed packaging, color life of fresh meats can be extended (Farber and Dodds, 1995). The light wavelength dependence of discoloration is more significant for oxymyoglobin in fresh meats than photo-oxidation of nitrosylmyoglobin in cured meats (Johnston, Knight, and Ledward, 1992).

### **2.3 Cured meat ingredients and chemistry**

Cured meats are defined by the USDA as “Meat soaked or injected with a brine solution to extend shelf life and, secondly, to impart the flavors of the curing agents”. Dry cured is defined as “Product labeled as "Dry Cured" shall not be injected with a curing solution or processed by immersion in a curing solution” (USDA, 2015). The basic dry cure process is to rub the meat with a mixture of dry Sodium Chloride (NaCl) and Potassium Nitrite ( $\text{KNO}_3$ ) and store in a similar dry mixture (Varnam and Sutherland, 1995). While the methods of wet and dry curing are different, the end result for ham is a characteristically pink color with salty flavor (Johnston, Knight, and Ledward, 1992; Varnam and Sutherland, 1995). Cured meats can also be cooked which results in the formation of a different pigment and color stability. All proteins can be cured, but in the United States & UK, pork is the most widely cured meat (Varnam and Sutherland, 1995). Ingredients in cured meats include NaCl, Nitrite, and curing adjuncts (polyphosphates, ascorbate, and erythorbate). The primary purpose of NaCl is flavor, water retention, and

preservation by lowering water activity. Nitrite is the active curing agent that acts as a micro-organism inhibitor, flavor agent, and is responsible for the formation of the characteristic color (Hunt et al., 2012). The maximum amount of sodium nitrite allowed to be added is 156 ppm (7 grams per 100 lbs. of meat) (Cassens, 1996). The FDA limits for sodium nitrite in meat curing is “not more than 200 parts per million in the finished meat product, and the amount of sodium nitrate cannot be more than 500 parts per million in the finished meat product (CFR - Code of Federal Regulations Title 21, Sec. 172.175, 2015). Nitrites and nitrates can be toxic to humans (particularly infants) causing the disorder Methemoglobinemia (blood disorder) (Agency for Toxic Substances and Disease Registry, 2014). Nitrites and nitrates have also been linked to diseases including leukemia, non-Hodgkin lymphoma, and many varieties of gastro-intestinal cancer (Healthy Child Healthy World, 2015). Water and leafy vegetables are a source of dietary nitrate, but cured meats are the major source of dietary nitrite (Hsu, Arcot, Lee 2009). For these reasons, the amount of free nitrite ingested should be minimized. Other curing adjuncts are added to improve water retention (polyphosphates), and improve color stability by acting as reductants (ascorbate and erythorbate) (Varnam and Sutherland, 1995). In the curing process, nitrite binds with the heme moiety of DMb, followed by a reduction of the bound nitrite to nitric oxide and simultaneous oxidation to NO-MMb (AMSA 2012). In anaerobic conditions and the presence of a reductant (erythorbate, ascorbate), NO-MMb is reduced to nitrosylmyoglobin (AMSA, 2012). When nitrosylmyoglobin is heated, the result is the formation of nitrosylhemochrome, which is responsible for the characteristic pink color (Varnam and Sutherland, 1995) (Figure 2.2), while 2 molecules of nitrite are needed for the formation of 1 molecule of nitrosylhemochrome (Hunt et al., 2012). The process leading to the formation of nitrosylhemochrome is not fully understood, however the basic reaction is when heated, nitrosylmyoglobin is denatured and detached from heme (Varman and Sutherland, 1995). Simultaneously a second molecule of nitrite is incorporated into the nitrosylhemochrome molecule complex (Killday et al., 1988). There is debate over whether di-nitrosylmyochrome or mono-nitrosylmyochrome is the resulting state. Current evidence indicates that the form is mono nitrosylmyochrome with 1 molecule of NO binding with the color producing heme group and the other molecule with the globin moiety (Hunt et

al., 2012). This process is aided by many different enzymatic and non-enzymatic systems (Bekhit and Faustman, 2005). This compound is sensitive to oxidation, which is catalyzed by light, resulting in the development of an undesirable brown colored metmyoglobin (Johnston, Knight, and Ledward, 1992).



**Figure 2.2** Interconversions of myoglobin redox forms in cured meats. *Courtesy of the American Meat Science Association*

The mechanism of meat curing and pigment formation is complex and the subject of many comprehensive reviews (Møller and Skibsted, 2002; Sebranek and Bacus, 2007). Skibsted describes the initial step as the reaction of nitrite with endogenous or added reductants (Johnston, Knight, and Ledward, 1992). The most significant reduction in the pH range of meat (5.6) is the reaction of dinitrogen trioxide ( $N_2O_3$ ) with the ascorbate ion (Johnston, Knight, and Ledward, 1992). It is hypothesized that up to seven intermediates are formed in this reaction (Johnston, Knight, and Ledward, 1992). None of these intermediates have been identified as free radicals, and diketogulonic acid is the only intermediate that has been isolated (Johnston, Knight, and Ledward, 1992). Varnam and Sutherland described the initial reaction in the pathway as oxidation of myoglobin to metmyoglobin by nitrite, coupled with a simultaneous reduction of nitrite to NO. This is

speculated to be followed by nitrite reacting with metmyoglobin to form nitrosylmetmyoglobin (an unobserved intermediate), which in turn undergoes a rapid auto reduction to a nitrosylmyoglobin radical cation. The final stage is further reduction of nitrosylmyoglobin radical cation to nitrosylmyoglobin (Varnam and Sutherland, 1995). Further research by Møller and Skibsted suggest that nitrite does not act directly as the nitrosylating agent, but does react to form  $N_2O_3$  that in the presence of reducing substances yields NO (Møller and Skibsted, 2002). Initial steps for ligand binding with the heme group are 1). movement of the ligand into the heme pocket, 2). displacement of a water molecule, 3). in plane movement of Fe to form a hex coordinate complex, and 4). Fe-ligand bond formation and stabilization (Møller and Skibsted, 2002). Favorability of the type of ligand bound (NO,  $O_2$  or CO) has been largely attributed to steric hindrance of the heme cleft, but electrostatic interactions and conformational changes in amino acid residues are also involved (Møller and Skibsted, 2002).

There is a controversy with the use of nitrite in curing. At a typical pH for meat, ascorbate reacts faster than secondary amines with the nitrosating agent ( $N_2O_3$ ). This competition is the primary mechanism proposed forth for the prevention of the formation of carcinogenic *N*-nitroso compounds (Johnston, Knight, and Ledward, 1992). In a perfect system, all nitrite can be accounted for as nitrate, nitrosylmyoglobin, gaseous nitrogen compounds, and residual nitrite (Varman and Sutherland, 1995). Transfer of NO to metmyoglobin is referred to as transnitrosation (Varnam and Sutherland, 1995). Nitrosation can also occur in non-heme proteins when nitrite reacts with tryptophyl to form nitroso derivatives. These derivatives play an important role in the oxidative stability of the cured meat color as the protein fraction of cured meats provide a source of nitroso groups (Varnam and Sutherland, 1995).

An important awareness in studying the cause of color change in cured meats is the  $a^*$  (from  $L^*a^*b^*$  color system) value and nitrosylheme concentration do not always correlate. No change in nitrosylheme concentration can still result in lower  $a^*$  values. In a study of sliced cured ham, packaging Oxygen Transmission Rates (OTRs) were found to have a significant effect on  $a^*$  scores, but did not significantly affect nitrosylheme concentration during storage (Li et al. 2012).

## 2.4 Cured meat pigment nomenclature

There are many different terms used for cured meat pigments without universal consistency (Table 2.2). Some terms are technically incorrect or can be interpreted differently, but nevertheless occur in the literature and a knowledge of the variation in nomenclature is helpful. Clarity can be gained by strictly using organic chemistry nomenclature. “For example, the nitroso prefix is technically incorrect for meat pigments because it is used to indicate nitric oxide attached to an organic structure such as a nitrosoamine. One can argue that myoglobin is an organic compound but in the case of meat pigments, nitric oxide is attached to the iron molecule in the heme, not the organic structure of the globin, and that makes nitrosyl the appropriate prefix to use for these pigments. Nitrosyl is used to indicate nitric oxide attachment to a transition metal such as iron (can also be chlorine, bromine, etc.).” (J. Sebranek, personal communication, December 15, 2014). Based on this, the cooked cured meat pigment nitrosylhemochrome is a better term. “Myochrome could also be a correct term, indicating the muscle pigment but if the heme group is detached from the globin in cooked meat as it is believed to be, then the more general “heme” term might be better suited to the cured pigment that probably includes some heme groups from hemoglobin as well as myoglobin. Uncooked cured pigment “nitric oxide myoglobin” is a proper term because the globin is still attached to the heme, and this reflects reaction of nitric oxide with myoglobin. However, this is also a nitrosyl bond similar to the cooked pigment so “nitrosyl myoglobin” would also be technically correct, though seldom used” (J. Sebranek, personal communication, December 15, 2014).



**Table 2.2** Terms used for cured cooked meat pigments (Promolex, 2012; Killday et al., 1988).

Cured meat terms
nitric oxide myochromogen
Nitrosylmyochrome
Nitrosohemochrome
Nitrosylhemochrome
Nitrosylhemochromogen
nitrosyliron (II). Protoporphyrin
Nitrosylheme
nitric oxide hemochrome

## 2.5 Intrinsic and extrinsic factors affecting raw meat color

The color of the final cooked product will be mainly dictated by the concentration and chemical nature of the starting raw meat material (Johnston, Knight, and Ledward, 1992).

There are many intrinsic and extrinsic factors that influence the chemical nature of the raw meats utilized. Extrinsic factors in raw meat include storage and processing conditions that impact the temperature / pH history post slaughter. The storage and processing conditions for the raw meat will strongly influence the chemical reactivity of the meat and subsequent pigment formation (Johnston, Knight, and Ledward, 1992).

Key factors include gender, age, seasonality, ante mortem stress, carcass weight, post-mortem conditions, post-mortem processing, and post-mortem age of the animal (Hunt et al., 2012; Johnston, Knight, and Ledward, 1992). For pork, there are multiple pre-harvest factors that influence color, including genetics, diet of the animal, and glycolytic potential (Mancini and Hunt, 2005). Depletion of glycogen in a live animal results in a translucent, dark, firm and dry (DFD) meat with high pH and high oxygen uptake, affecting the light scattering properties of the meat (MacDougall 1986). The presence of natural antioxidants like vitamin E in the diet can affect oxidative and color stability (Møller, Weber and Bertelsen, 1999). Vitamin E delays the formation of secondary peroxidation products and improves color stability by scavenging free radicals (like peroxy which are lipid peroxidation process promoters) (Suman et al., 2014). Evaluating

several sire lines, Brewer et al. reported that differences were found in pinkness and  $a^*$  value (Brewer et al., 2004). Pigs with halothane allele in place of a normal allele were found to produce the most pale, soft, and exudative muscle properties (McPhee and Trout, 1995). Other intrinsic factors include pH, muscle type, areas within the muscle, muscle fiber composition, myoglobin concentration, water holding capacity and microbial load (Hunt et al., 2012). All of these factors have a critical effect on the meats use of oxygen, and the meat's ability to reduce MMb (Hunt et al., 2012). Beef color development measured using  $L^*a^*b^*$  values were found to be lower with a  $pH \geq 6.1$  than with a group with a  $pH \leq 6.1$  (Abril et al., 2001). Comparing the ventral and dorsal portions of raw steaks, the ventral portion was found to be lighter, redder, and more yellow (as detected by a Hunter Miniscan XE  $L^*a^*b^*$ ) (Lee et al., 2008). Myoglobin structure is similar in all animals, but minor differences between and within species may account for observed visual differences and color stability in meats (Varnam and Sutherland, 1995). The muscles of a hare for example have more myoglobin than a rabbit (Johnston, Knight, and Ledward, 1992). Red meats (like beef) have two to three times more myoglobin than white meats (like poultry) (Johnston, Knight, and Ledward, 1992). Beef contains 4-10 mg of myoglobin per gram of wet tissue, while pork has 3 mg of myoglobin per gram of wet tissue (Varnam and Sutherland, 1995). Ultimately a systems approach that includes genetics, production factors, pre and post issues combined with defined packaging and storage temperature parameters is the best method for controlling the color stability of the raw meat (Suman et al., 2014).

## **2.6 Cured meat product formulation effect on end color**

The quantity and type of ingredients added have an influence on the end color of the cured meat. The more nitrite added, the higher the intensity of reddish / pink color (Møller, Weber and Bertelsen, 1999), unfortunately high levels of nitrite can lead to a bright green discoloration known as nitrite burn (Varnam and Sutherland, 1995). Color fading has been shown to be a partially reversible process suggesting residual nitrite and excess ascorbate play a role after initial color formation (Johnston, Knight, and Ledward, 1992). Nitrite can be added to the formulation directly or indirectly through the packaging. In a study of fresh and frozen beef, nitrite embedded films increased redness,

but results were also dependent on the muscle type and age at the time of packing (Suman et al. 2014). The amount of residual nitrite in the end product is important as it can 1) provide a source for additional nitric oxide production, 2) act as an antimicrobial (Shahidi and Pegg, 1992), and 3) act as an antioxidant. The pH of the product and other added ingredients has been demonstrated to affect the residual nitrite in the product. Wieners formulated with mechanically separated turkey and sodium tripolyphosphate resulted in a higher pH product, and retained more residual nitrite as a result (Kilic, Cassens and Borchert, 2002). Cooked beef roasts manufactured from meat with a high pH (6.5) resulted in redder product when compared to meat with a pH of 5.5 (Swan and Boles, 2002). Addition of lactate with nitrite during meat curing is hypothesized to result in a more complete reduction of nitrite to nitric oxide and increased color development (McClure et al., 2011). Addition of malate to beef mitochondrial and cytoplasmic isolates at a pH 7.2 increased reduction of metmyoglobin. The combination of malate and lactate together was equal or greater than malate alone in reducing metmyoglobin via NADH regeneration (Mohan et al. 2010).

The end color is also affected by the presence of reducing factors in the meat such as enzymatic co-factors and sulphide groups in peptides (that can act to reduce nitrite) (Møller, Weber and Bertelsen, 1999). These factors can be overcome with the addition of reducing additives like ascorbate (Møller, Weber and Bertelsen, 1999). The rate of formation of nitroso compounds was demonstrated to increase in the presence of high chloride (Cl) (Johnston, Knight, and Ledward, 1992). The effect of high salt content (NaCl) during curing may also contribute to the transformation of nitrous acid ( $\text{HNO}_2$ ) into nitrosyl chloride which is more reactive than  $\text{N}_2\text{O}_3$ , but not as reactive as  $\text{NO}^+$  (Johnston, Knight, and Ledward, 1992).

The type of manufacturing formulation (for example lean deli meat compared to summer sausage) will also influence the total myoglobin present and the final color. Cured ham has a higher myoglobin content compared to sausage simply because of the greater amount of fat in the latter (Møller, Weber and Bertelsen, 1999). The formation of heme containing pigments in fermented sausages like salami are the same pathway as other nitrite cured meats, however because of the low pH of the formula, the pigment responsible for color is nitrosylmyoglobin regardless if the product has been heated

(Varnam and Sutherland, 1995). With the amount of  $H_2O_2$  producing bacteria and greater potential of fat rancidity in fermented sausages, the risk of discoloration is greater because it has a more favorable environment for metmyoglobin formation (Varnam and Sutherland, 1995).

Water content has also been demonstrated to affect the end color of the product, particularly after storage. In a study of cooked pork ham using several cooking techniques (1) wet air cooking, 2) dry air cooking, 3) water cooking) the moisture content of the end products varied, and the quality of the products after storage differed on attributes of hardness, texture, and color (measured by  $a^*$  and  $b^*$ ) (Cheng and Sun, 2004).

## **2.7 The impact of other sandwich components**

The two most common ingredients found in a sandwich with cured meat are bread and cheese. With these components, three factors potentially influencing cured meat color are 1) effect on overall pH, 2) end water content, and potential trapped oxygen in the sandwich. Bread is very porous and the pore structure is the result of one larger interconnected structure that is open to the atmosphere (up to 99% connected) (Wang, 2014). The bread structure creates a potential for trapped oxygen that can be shielded from the vacuum process unless a significant vacuum is applied for a long period of time. A typical pH range of plain breads is 4.5 to 5.5. Pasteurized processed cheese has a maximum pH value of 6, while cured meats can vary from 5.5 to 6.5. Establishing the initial pH of each component and establishing the end pH of the sandwich system should be considered for potential impact to cured meat color. Also with a reported water activity in bread of 0.91 to 0.95, and meats 0.95 to 0.97, the potential exists for the system to equilibrate, altering the moisture content in the meat. Cured meats of different moisture contents have been demonstrated to vary in color (Cheng and Sun, 2004).

## **2.8 Modified Atmosphere Packaging (MAP)**

A simple definition of MAP is when a food is packaged in an atmosphere that is different from normal atmospheric composition (78.08%  $N_2$ , 20.96%  $O_2$ , 0.03%  $CO_2$ ). This is

accomplished by applying a vacuum to the product in the package followed by gas replacement with the desired gas composition then sealing. Packaging material with a high gas barrier is used to control the diffusion of gas in and out of the package after sealing (Farber and Dodds, 1995). The amount of vacuum applied and gas replaced can vary. Anderson et al. found that when comparing sliced ham in 1) 99% vacuum, 2) carbon dioxide flushing followed by 90% vacuum, and 3) a slight overpressure in combination with CO<sub>2</sub> flushing eliminated the need to store the product in dark storage for 4 days prior to exposure to illumination to avoid discoloration (Anderson et al., 1990). Vacuum packing and Controlled Atmosphere Packaging (CAP) are also considered types of MAP techniques (Farber and Dodds, 1995). In vacuum packing, the product is placed in high barrier film, air is evacuated, and the packaging is sealed (Farber and Dodds, 1995). The difference between MAP and CAP is that in CAP, the gas atmosphere is continually controlled throughout the shelf life, where MAP is initially controlled, but then changes based on the respiration of the food and the gas permeability of the film (Arvanitoyannis, 2012). Another technology similar to MAP is Sous Vide which is translated “under vacuum”. Product is packaged under vacuum in hermetically sealed bags, then cooked or heated, cooled and refrigerated (Farber & Dodds, 1995). The storage life of a wide variety of foods including Ready-To-Eat (RTE) foods and cured meats can be extended using MAP (Farber & Dodds, 1995). In addition to shelf life extension, other benefits of MAP include increased distribution reach, improved cost due to larger scale production, maintaining freshness with minimal preservatives, and better sales appeal of product due to attractive color and presentation (Farber & Dodds, 1995). Each food product has its own deterioration mechanism that requires consideration when selecting the appropriate packaging method and parameters. Considerations include intrinsic factors such as pH, respiration rate, and chemical composition of the food in addition to extrinsic factors such as storage temperature and relative humidity during storage (Arvanitoyannis, 2012). Sliced cooked pork shoulder achieves a 28 day shelf life in an 80% N<sub>2</sub> + 20% CO<sub>2</sub> MAP package. Pastirma (air dried cured beef) achieves a 150 day shelf life in a 50% N<sub>2</sub> + 50% CO<sub>2</sub> flushed package (Arvanitoyannis, 2012). Without the prerequisite of MAP, product discoloration can precede rapidly turning cured meat color brown within 24 hours of refrigeration (Anderson et al., 1990). However, the

widespread use of MAP for cured meats has also generated issues in color stability under illumination in retail display (Møller et al. 2002).

Careful consideration should be given to the type of MAP equipment selected. The two main categories of MAP equipment are pillow wrap and chamber (Arvanitoyannis, 2012). Chamber machines include thermoforming and preformed container machines. The difference in model types is the preformed tray model is not capable of forming a tray inline. Both varieties work by evacuating the pouch using vacuum and gassing back followed by a hermetic seal. Pillow wrap machines work by passing the flexible film through a forming tool to form a tube around the product. A lance is used to gas flush the package and displace the normal atmosphere. The product is sealed by crimp sealing the fin and ends of the package (Arvanitoyannis, 2012). There are several challenges that manufacturers need to consider with MAP product including removing head space oxygen, packaging leaker detection, and sustainability. Removing head space oxygen can be accomplished with vacuum pack, but only if vacuum pack has an acceptable appearance to the consumer. In the case of a ham nugget, this would be acceptable, in the case of a sandwich; it would not meet consumer expectation. Oxygen scavengers are an alternative, but a key concern is that it is a foreign object, and it can be mistakenly eaten. A leaker detection system is critical. Methods can include visual inspection, pressure decay systems, vision systems to automatically detect, and thermal systems that inspect after sealing.

## **2.9 Photo oxidation and Thermal oxidation**

The pigments of cured meat are susceptible to oxidation (Johnston, Knight, and Ledward, 1992). The rate of oxidation of nitrosylmyoglobin and other heme pigments decreases with lower oxygen partial pressure, and increases with light (Johnston, Knight, and Ledward, 1992). Oxidation of nitric oxide myoglobin is prevented if oxygen is excluded, but proceeds rapidly in light if oxygen is present (MacDougall, 1982). In thermal oxidation, the conversion of nitrosylmyoglobin into metmyoglobin in oxygen saturated environments follows first order kinetics, has a rate constant of  $3 \times 10^{-4} \text{ s}^{-1}$  at  $26^\circ\text{C}$  and energy of activation of  $92 \text{ kJ mol}^{-1}$  (Johnston, Knight, and Ledward, 1992). The stoichiometry of the reaction is one to one (Johnston, Knight, and Ledward, 1992). Heat

denaturing of nitrosylmyoglobin increases the activation barrier for the reaction and improves the color stability (Johnston, Knight, and Ledward, 1992). At room temperature, discoloration of fresh meats is mainly due to thermal oxidation (Bertelsen and Skibsted, 1987). Thermal oxidation of cured meat in dark refrigeration is very slow, but accelerates in light (Anderson et al., 1990). Under frozen conditions, thermal oxidation is suppressed, but photo-oxidation can occur in the presence of light (Bertelsen and Skibsted, 1987).

The mechanism of discoloration of cooked cured meat in a low oxygen MAP package in the presence of light is photo-oxidation. Photo-oxidation occurs when light absorption of heme protein causes nitrosylmyoglobin to dissociate into nitric oxide and myoglobin (Johnston, Knight, and Ledward, 1992). The precise mechanism of photo-oxidation is not known (Johnston, Knight, and Ledward, 1992; Sun et al., 2009). The oxidative products are metmyoglobin and nitrate and the rate of oxidation is very dependent on oxygen partial pressure and temperature, but not on pH, ionic strength and solution viscosity (Anderson and Skibsted, 1992). The stoichiometry for photo-oxidation was found to be larger than 1 to 1, indicating that the reaction mechanism is different from thermal oxidation, and providing insight on why a small amount of oxygen can cause significant discoloration (Møller, Bertelsen and Skibsted 2002; Møller, Nannerup and Skibsted, 2005).

Several proposed mechanisms exist for photo-oxidation. A bimolecular reaction between nitrosylmyoglobin (MbNO) in an electron excited state, oxygen, and a transition state with a partly dissociated nitric oxide molecule was suggested (Anderson and Skibsted, 1992). The presence of an interfering radical species nitrosyldioxy-radical (ONOO-) has also been suggested capable of initiating lipid oxidation (Munk et al., 2010). It was suggested that the formation of the radical ONOO- occurred in the heme cavity and was in competition with NO- rebinding, and O<sub>2</sub> to a lesser extent, binding to form MbFe<sup>II</sup>O<sub>2</sub>. Subsequent oxidation of MbFe<sup>II</sup>O<sub>2</sub> was expected to lead to formation of a highly reactive peroxynitrite intermediate (ONOO-), but using mass spectrometry, ONOO- was not detected (Munk et al., 2010).

Other current proposed mechanisms suggest that photo oxidation under aerobic conditions is the result of two parallel reactions (Munk et al., 2010). The first reaction is

light induced. In the excited state, ONOO<sup>-</sup> (nitrosyldioxygen radical, which is mildly oxidizing) is formed. The second is the result of NO<sup>-</sup> trapped in the cavity of the nitrosylmyoglobin and nitric oxide moiety (MbFe<sup>II</sup>NO) and is dependent on oxygen with a similar mechanism to thermal oxidation. Photo-oxidation of nitrosylmyoglobin in a 20% CO<sub>2</sub> + 80% N<sub>2</sub> gas flush with residual oxygen levels of 0.1%, 0.5%, and 1.0% was found to depend linearly on the amount of oxygen present for both visible (436 nm) and UV light (366 nm) (Møller, Bertelsen and Skibsted, 2002). In thermal autoxidation, the mechanism follows a first order reaction rate. The reaction is dependent on the temperature and oxygen partial pressure. In contrast, photo oxidation was found to be only moderately dependent on the wavelength of light, is proportional to the partial pressure of oxygen and increased in viscous solutions (Anderson and Skibsted 1992). The precise structure of oxidized nitrosylhemochromogen is unknown (Sun et al., 2009). Sun et al. suggest that the NO<sup>-</sup> group might not detach from the iron porphyrin (Sun et al., 2009).

## 2.10 Gas composition

There are a variety of gases that can be used to replace the standard atmosphere in a package. Different combinations have been found to benefit certain product types. For fresh red meat, a high oxygen content (>80%) or carbon monoxide (CO) (0.4%) helps to preserve a cherry red product (Mancini and Hunt 2005). For cooked meats in gas flushed systems, 20 – 40 % CO<sub>2</sub> combined with 60-80 % N<sub>2</sub> has been shown to be effective in extending shelf life (Church and Parson, 1995). Inert gases nitrogen and argon provide a good replacement options, however economics is an important consideration when selecting. The cost of argon (Ar) is six times the cost of nitrogen (resulting in a cost increase of \$.004 per unit for a wedge sized sandwich). Research on the benefits of argon in MAP is limited, however in a study of fresh raw turkey, “The Ar-CO<sub>2</sub> mixture was more efficient in delaying flora development than CO<sub>2</sub>-N<sub>2</sub> with 1 log difference on the 25th day of storage, for total psychotropic counts, total anaerobic counts, and *Brochothrix thermosphacta*. The presence of Ar on gas mixtures did not seem to have any additional protective effect on lipid turkey meat oxidation. (Fraqueza and Barreto, 2009). Carbon dioxide is the one gas that has been implicated to provide microbiological benefit in the



form of dissolving into the product to form carbonic acid lowering surface pH (Arvanitoyannis, 2012). The optimal concentration of CO<sub>2</sub> has not been established and results are product specific (Farber and Dodds, 1995). In studies of fresh pork loin, product packaged in high CO<sub>2</sub> environments did not hinder microbial growth when compared with other gas treatments (Viana, Gomide and Vanetti, 2005). Iberian ham performed best for color (*a*\* score) in 80% N<sub>2</sub> + 20% CO<sub>2</sub>, but lower overall *a*\* values were found over 120 days for 60% N<sub>2</sub> + 40% CO<sub>2</sub>; 70% N<sub>2</sub> + 30% CO<sub>2</sub>; 70% argon + 30% CO<sub>2</sub> (Parra et al., 2009). Fresh pork loin color performance was best in a 99% CO<sub>2</sub> + 1% CO environment when compared to atmospheres of 100% CO<sub>2</sub>, 100% O<sub>2</sub> & 100% CO over 20 days of storage. The *L*\* and *a*\* values remained similar to fresh meat values using this combination (Viana, Gomide and Vanetti, 2005). There has been speculation as to whether carbon dioxide plays a role in both the photo-oxidation and autoxidation mechanism. However, using horse heart to synthesize nitrosylmyoglobin, solutions saturated with 0, 20 and 90% CO<sub>2</sub> found quantum yields of metmyoglobin formation to not show a dependence on CO<sub>2</sub> levels (Møller, Nannerup and Skibsted, 2005). Pasteurized ham in an 80% N<sub>2</sub> + 20% CO<sub>2</sub> blend with a 1:3 head space ratio and 0.1 to 0.5 % headspace oxygen helped avoid light induced discoloration (Møller et al. 2000). In a study of sliced dry-cured ham, redness values (Hunter *a*) were not affected by time (0, 21, 56 days) or the packaging system (vacuum, 100% N<sub>2</sub>, 80/20% N<sub>2</sub>/CO<sub>2</sub>). Lightness (measured as Hunter *Lab*) was found to be more stable with 20% CO<sub>2</sub> + 80% N<sub>2</sub> (Garcia-Esteban, Ansorena and Astiasaran, 2004). Color scores for *a* value ranged from 19.36 to 22.53 (Garcia-Esteban, Ansorena and Artiasaran, 2004). For Roast beef packaged under vacuum as compared to 100% CO<sub>2</sub> flush, it was found that the vacuum packed product was rejected after 3 weeks at 3 C° compared to 10 weeks using the 100% CO<sub>2</sub> replacement (Penny, Hagyard and Bell, 1993).

## **2.11 Extrinsic factors influencing cured meat color**

Packaging and storage conditions are significant contributors that affect cured meat discoloration. Critical packaging and storage factors include 1) percent residual oxygen, 2) product to headspace volume ratio (P/H volume ratio), 3) oxygen transmission rates (OTR), 4) temperature, and 5) light intensity (Møller et al. 2002; Nannerup et al. 2004).

The complexity of the interaction of these factors justifies evaluating them simultaneously (Møller et al. 2002; Nannerup et al. 2004). In a multifactorial design looking at 1) percent residual oxygen, 2) OTR, 3) product to headspace volume ratio, 4) illumination level, and 5) nitrite level, Møller et al. found significant effects in all main factors on the tristimulus  $a^*$  color scores (Møller et al. 2002). Similar evaluations of these factors in combination have shown that percent residual oxygen and P/H volume ratio need to be considered together (Nannerup et al. 2004).

### **2.11.1 Residual oxygen and P / H volume ratio**

Low residual oxygen in the package is of paramount importance. Anderson et al. found that the color of sliced, vacuumed packed ham improved remarkably with an immediate four day refrigerated dark storage. The conclusion was the time in dark storage allowed for depletion of the oxygen from post mortem processes and microbiological activity (Anderson et al., 1988). In a study of sliced cured ham to optimize color stability during packaging and retail display, Møller et al. concluded that the interaction between measured oxygen percentage in the head space and the product to headspace volume ratio was critical. A low head space oxygen wasn't enough if the headspace volume is large, sufficient oxygen will be available for discoloration to take place (Møller et al. 2002). A volume head space ratio of 1:1.3 and measured oxygen content of 0.1% resulted in an  $a^*$  value of 5.6, where a ratio of 1:4.9 at the same oxygen content resulted in an  $a^*$ -value of 2.8. This effect was attributed to the total oxygen available in the volume space (Møller et al. 2002). In a similar study of cooked cured ham, Nannerup et al. found that changing the product head space (P/H volume ratio) ratio from 1:1 to 1:3 resulted in  $a^*$  score reduction of 24% (Nannerup et al. 2004). Of all the critical parameters investigated in this study which included percent residual oxygen, P/H volume ratio, temperature, light intensity, and OTR rates, the interaction of percent residual oxygen and P/H volume ratio was found to increase the degree of discoloration the most (Nannerup et al. 2004). Using sliced pasteurized ham in Modified Atmosphere Packaging (MAP), a threshold range of 0.1 – 0.5% for residual oxygen in the headspace was established depending on other packaging parameters (Møller, Weber and Bertelsen, 1999). In a study of the effects of high pressure treatment and residual oxygen percentage (<0.1%, 0.1-0.2%, 0.2-0.3%)

on the color stability of minced cured restructured ham at different levels of drying (20%, 50%), pH, and NaCl levels, Bak et al. concluded that raw meat pH, salt content, and residual oxygen had varying effects on the stability of the red color (measured using a Chroma meter). With low salt content, 0.05% O<sub>2</sub> had a smaller decrease in delta  $a^*$  over time compared to 0.25% O<sub>2</sub> with low salt content. In the case of high salt content, increasing O<sub>2</sub> levels from 0.05% to 0.25% did not decrease  $a^*$  values (Bak et al., 2013). This underscores the complexity and need of reviewing multiple factors simultaneously.

### **2.11.2 Oxygen Transmission Rates (OTR)**

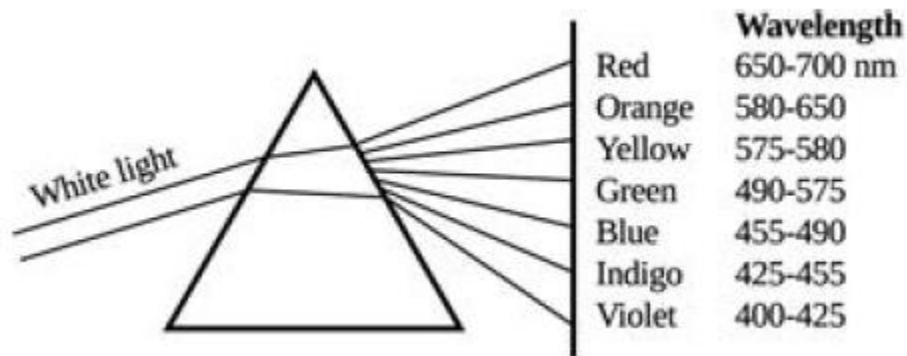
It is vital for film to have an adequate OTR. In a study of sliced ham, the combination of a low OTR ( $<4\text{cm}^3/\text{m}^2/24\text{ h/atm}$ ), high initial vacuum ( $>99\%$ ) and cold dark storage until residual oxygen had been consumed (4 days) was found to prevent discoloration (Anderson et al., 1988). In another study of sliced ham,  $a^*$  values of samples in a film with an OTR of  $60\text{ cm}^3/[\text{m}^2 \times \text{day} \times \text{atm}]$  were significantly lower than those with an OTR of either 30 or  $1.5\text{ cm}^3/[\text{m}^2 \times \text{day} \times \text{atm}]$  (Li et al. 2012). In this study, storage time also had a significant effect on  $a^*$  values in the illumination group. Days 1-7 saw an increase in  $a^*$  values, followed by a decrease during days 7-14, and then another increase from 15-21 days (Li et al. 2012). In a study of dry salami, product had a greater retention of redness as the OTR of the film decreased from  $90\text{ cc O}_2/\text{m}^2/24\text{ hr}$  to  $<11\text{ cc O}_2/\text{m}^2/24\text{ hr}$  (Yen et al. 1988).

### **2.11.3 Display temperature**

The display temperature has a significant impact on color stability (Hunt et al., 2012). The reported display temperatures for meat color studies has typically been  $>4.5\text{ C}^\circ$ , but most retail coolers run higher than this, and the defrost cycle for most refrigerators will exceed this (Hunt et al., 2012). This is significant as reaction rates increase with increasing temperature (Chang, 1991). Nannerup et al. found the difference in color stability between 5 and  $10^\circ\text{ C}$  in a model system with ideal residual oxygen, P/H, OTR and light intensity did not vary significantly, but noted that microbial activity is affected in this range (Nannerup et al. 2004).

## 2.12 Light and color interpretation

A basic understanding of the physics of color and light is necessary to understanding what role the light source plays in meat discoloration. While human color perception is reasonably uniform unlike other senses, there are individual differences between people, and the spectral sensitivity of the human eye is not equal to a spectrophotometer (Hui, 2007). The necessary components for a color to be detected are an object, its surroundings, and a detector (Hunt et al., 2012). While a detector can be an instrument, the most practical example of a detector is the human eye. The eye consists of cornea, pupil, iris & lens. Light comes through the pupil, and is then focused by the lens to the retina, with the iris regulating how much light comes through (Hui, 2007; Hunt et al., 2012). The retina is composed of rods and cones. Rods detect light and dark, cones detect the light spectra in primary colors red, blue & green (Hui, 2007; Hunt et al., 2012). This information is sent to the brain via the optic nerve for interpretation. In the electromagnetic spectrum, the human eye is only capable of detecting wavelengths of light in the visible spectrum (380-770 nm) (Hunt et al., 2012). If the wavelengths being reflected are not in the visible spectrum, no color is interpreted (Wrolstad and Smith, 2010). Different wavelengths of light yield a different perception of the color (Figure 2.3).



**Figure 2.3** White light splitting into component wavelengths. *Courtesy of the American Meat Science Association.*

Color ultimately is the mixture of three attributes 1) hue, 2) lightness (also referred to as value), and 3) saturation (also referred to as Chroma). Hue is what we instinctually

referred to as “color” but really refers to a color wheel including all variations of color (Wrolstad and Smith, 2010). Lightness is how bright (or dark) the object is and saturation is a measure of vividness. When light strikes an object, the light is either reflected back to the observer for color interpretation, or it is retained by the object (Hunt et al., 2012). There are several factors that influence interpretation including light source, intensity of the light source (lumination), observer differences, texture of the object (rough vs. smooth) and the angle of reflection (Hunt, et al., 2012). In the instance of a rough surface, the reflection back from the surface is called a diffuse reflection. Light in this instance is scattered. Light from a smooth source is specular reflection, and the light we see is a direct reflection from what is in front of you (Serway and Faughn, 1989). Iridescence in meat (particularly beef) is an example of reflection from a rough surface that the eye interprets as a shiny rainbow like appearance (Hunt, et al., 2012). The angle which the object is viewed from and the condition of the surface are important because of how it will reflect light. A glossy surface vs. flat may change the interpretation of the light. As it relates to meat color interpretation, AMSA recommends that background lighting should be avoided with a preference of an overhead light with a standardized intensity (Hunt, et al., 2012). Also recommended is a constant viewing angle of 45° with reduced glare (Hunt, et al., 2012). Best practices for evaluating color are important as there are many factors that can influence the end interpretation of color. The reality of the store display is that products will be placed in a variety of positions throughout the shelf life with different background lights and viewing angles that will produce a variety of perceptions. Light sources have different spectral power outputs. A balanced output would be one with equal output of different wavelengths. As a reference, fluorescent bulbs have greater outputs at wavelengths of approximately 420 nm & 540 nm (Hunt et al., 2012). Two important factors to consider when examining the light bulb as a light source is illumination level and spectral power distribution (Sylvania, 2014). Illumination is the light energy in contact with a given unit area. The amount of energy that reaches an object is  $E$  (radiant energy) =  $C$  (intensity of the source) /  $d^2$  (distance squared). Intensity can be expressed in units of candles. Distance is expressed by feet, so Energy is expressed as a foot-candle (Freeman, 1990). A foot candle is defined as 1 lumen per square foot. The term lux is a metric standard unit for defining illumination. It is equal to

one lumen per square meter and is a measure of intensity. As a point of reference, 500 lux is the equivalent of office lighting, and a 1000 lux is the equivalent of an overcast day (Kitsinelis, 2011). The human eye is sensitive to different wavelengths of light. The concept of photopic spectral luminous efficiency function ( $V(\lambda)$ ) (i.e. sensitivity to how the human eye sees various wavelengths) helps to relate Watts to Lux. Based on this concept, .0029 Watts of green light ( $\lambda = 510$  nm,  $V(\lambda) = 0.5$ ) will provide an illuminance of 1 Lux, .0015 Watts of yellow light ( $\lambda = 555$  nm,  $V(\lambda) = 1$ ) provide 1 Lux, and .015 Watts of red light ( $\lambda = 650$  nm,  $V(\lambda) = 0.1$ ) provide 1 Lux (Sylvania, 2000). Table 2.3 provides a definition of light sources and corresponding Watts and Lux values.

**Table 2.3** Types of light sources (Kitsinelis, 2011)

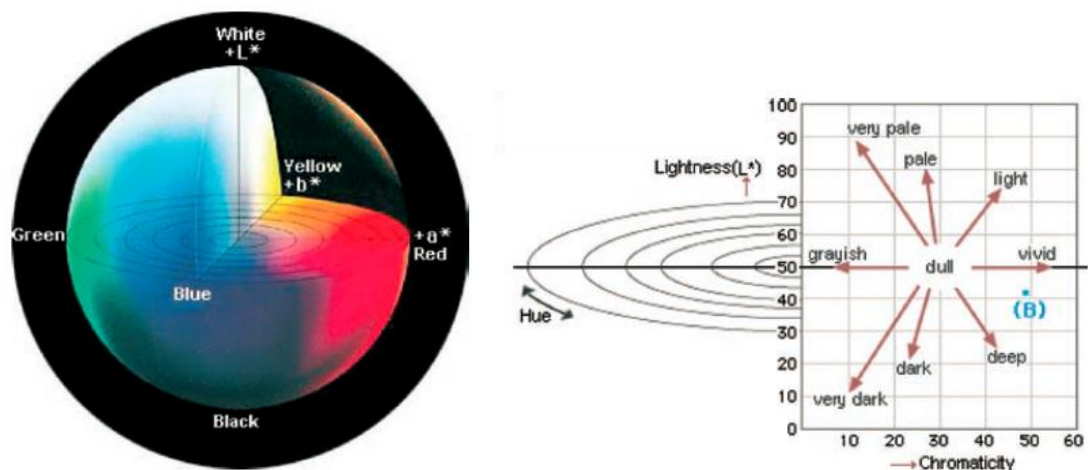
Bulb type	Basic function	Watts
incandescent light bulb	Produces light when a wire filament is heated to a high temperature . The filament is protected from Oxidation with inert gas or a vacuum.	15-1000
Fluorescent lamp	An electrical current excites mercury vapor which produces ultraviolet light that causes the phosphorous coating to glow.	5-125
LED (Light Emitting Diode)	Voltage is applied to leads and electrons recombined with electron holes within, releasing energy as photons.	0.1 - 7

The illumination level is related to perceived power of the source while the spectral power distribution is related to the strength of the wavelengths being emitted. The spectral emissions from a light source come from the spectrum emitted by the phosphor coating, and the spectral lines that come from the mercury arc in the bulb (Sylvania, 2014). Sylvania offers Spectral Power distribution curves that represent the total power output of its bulbs between 350 nm to 750 nm. While the human eye does not pick up all wavelengths (the eye is sensitive to specific wavelengths), it does offer insight that not all bulb types have the same power output across all wavelengths. There is literature that supports the use of alternate light bulbs from fluorescent. Promolux promotes lamps that have a better balance visible spectra, using red blue wavelengths, and minimizing potential harmful yellow and green wavelengths found in fluorescent bulbs (Promolux, 2012). Most food displays use fluorescent lamps because of the efficiency, low energy consumption and lower heat production (MacDougall, 1986).

## 2.13 Color measurement

There are two instrumental options for measuring color 1) tristimulus chromameter, 2) spectrophotometers (Hunt et al., 2012). They differ with how the sample is presented, size of the viewing area, portability, and the ability to measure by transmittance versus reflectance (Wrolstad and Smith, 2010). A tristimulus chromameter only measures tristimulus values (CIE  $L^*a^*b^*$ ) and has a set illuminant and observer combinations (Hunt et al., 2012). Spectrophotometers are more complex providing a broader spectral analysis in intervals of 1 to 10 nm, and offer several illuminant / observer combinations (Hunt et al., 2012).

The Commission Internationale de l'Eclairage (CIE) is the key international organization for color & color measurement. First established in 1931, the CIE created color standards for three standard CIE illuminants (Wrolstad and Smith, 2010). This includes Illuminant C which represents overcast daylight, Illuminant D65 which is average daylight plus ultraviolet wavelength region, and Illuminant A which represents incandescent light (Wrolstad and Smith, 2010). In 1942, the Hunter color standard was published where  $L$  indicated lightness,  $a$  indicated red / green coordinate, and  $b$  indicated yellow / blue coordinate (Figure 2.4) (Wrolstad and Smith, 2010).



**Figure 2.4** *Lab color space reprint from Konica Minolta*

In 1976, the CIE recommended  $L^*a^*b^*$  color scale as an improvement on color spacing. This created a uniform color scale where the distances between colors were plotted on a

3D scale, with  $L^*$  correlating to light & dark,  $a^*$  to red and green, and  $b^*$  to yellow and blue. The color sensitivity of the eye changes with the angle of view. The CIE defined the standard observer using a  $2^\circ$  viewing angle in 1931. In 1964, an additional viewing angle of  $10^\circ$  was added (Hui, 2007).

In the study of minced cured restructured ham using a spectrophotometer, the shape of the reflectance curves did not show evidence of other types of myoglobin, supporting the suggestion that the decrease in redness is the result in some structural change that is not detectable by a spectrophotometer (Homgaard Bak et al., 2013).

## **2.14 The effect of Ultraviolet (UV) light, visible light, lumination level and light type on meat**

Literature regarding the impact of light source and intensity on meat color is inconsistent. Many of the studies agree that discoloration is proportional to light level and exposure time, but there is no consistency on whether ultraviolet (UV) or the visible spectrum is more damaging (Sylvania, 2014). The amount of infrared power should be minimized because it represents heat (Sylvania, 2014). The type and of meat (fresh or processed) and state of the meat (frozen or refrigerated) is important (Sylvania, 2014). Processed meats (including cured) require protection from oxygen, while fresh meat can produce a desirable color in the presence of oxygen (Hunt et al., 2012). There are many studies that examine the effects of light on both cured and fresh meats, but often the focus of the study isn't on the bulb source alone. It is often coupled with packaging conditions such as low OTR, variation in residual oxygen, and vacuum methods.

## **2.15 Light source and intensity**

In a study of fresh beef, pork, and ground turkey; beef semimembranosus steaks and other beef products had less discoloration under LED lights compared to fluorescent (Steele, 2011). In the same study, pork chops under LED had higher  $L^*$  values, and lower  $a/b$  ratio (Steele, 2011). In a study of refrigerated ( $2^\circ\text{C}$ ) fresh pork packaged in 80% oxygen and 20% carbon dioxide under lighting conditions of 1) dark, 2) standard fluorescent bulb, 3) low-UV color balance bulb and 4) standard fluorescent bulb with UV filter, a significant decrease in  $a^*$  value occurred under standard fluorescent bulbs



decreasing shelf life from 12 to 8 days based largely on discoloration (Martinez, Cilla and Beltran 2006). The standard fluorescent bulb with a UV filter helped, but the low UV lamp was found to not improve shelf life for color (Martinez, Cilla and Beltran 2006). Fresh pork longissimus loins were evaluated at 0, 7 and 14 days under seven different bulb types and scored by a trained color panel. The panel found the poorest color correlated with Cool White fluorescent bulbs, and the most desirable color under Sylvania® Gro-lux® wide spectrum fluorescent bulbs (Kropf, Hung and Hunt, 1987). Fresh beef steaks using MAP packaging with a gas mixture of 70% O<sub>2</sub> + 20% CO<sub>2</sub> + 10% N<sub>2</sub>, under lighting with no or low UV radiation led to a significant delay of meat spoilage as determined by surface color (*a*\* and MMb percentage) (Djenane et al., 2001). The study compared 1) standard fluorescent, 2) fluorescent with a UV filter, 3) low UV color balanced lamp, and 4) darkness. The shelf life under 2 and 3 increased to 22 to 28 days compared to only 12 days under fluorescent lighting. The study measured surface color using a reflectance spectrophotometer and a trained 6- member color panel (Djenane et al., 2001). Frozen minced beef (product temperature -18 C°) was found to have significantly improved color stability (measured with tristimulus chromameter) under fluorescent lighting by using an ultraviolet barrier in the packaging that excluded light under 350 nm (Anderson, Bertelsen and Skibsted, 1989). The protection from discoloration as a result of the UV barrier is predicted to be effective up to a temperature of 5°C. Below the temperature of 5°C, the rate of light-induced photo oxidation exceeds thermal oxidation. Because at lower temperatures photo-oxidation is the dominate discoloration reaction, use of a UV barrier improves product color. Above 5°C, the relative rate of thermal oxidation exceeds that of photo-oxidation rendering UV color protection ineffective (Anderson, Bertelsen and Skibsted, 1989). In a study of sliced cooked cured ham in vacuum packaging, Li et al. found that illumination had no significant effect on the *a*\* value across the conditions of 1000, 200, and 0 lux through 28 days. The *a*\* values in this study varied, increasing during days 1-7 of storage and then decreasing over days 7-14 in all three lamination conditions. The differences in *a*\* value between lamination conditions wasn't significant (Li et al., 2012). The illumination levels and packaging OTR also did not affect nitrosylheme concentration (Li et al., 2012). In a study of sliced cooked ham, Haile et al. reviewed the effect of light (1000 lux) on color

and lipid stability under the packaging conditions of wrapped in foil (in the dark), wrapped in foil (exposed to light) and gas flush (50% gas back of 30% CO<sub>2</sub> + 70% N<sub>2</sub>; and 75% gas back of 30% CO<sub>2</sub> + 70% N<sub>2</sub>), concluding that light had a detrimental effect on redness ( $a^*$ ) over time (40 days) (Haile et al., 2013). The study also confirmed that light had a detrimental effect on color stability in the form of higher  $L^*$ , MetMB%, and nitrosomyoglobin concentration (Haile et al., 2013). The non-gas flushed foil in dark storage did not show significant discoloration, while the foil wrapped exposed to light decreased steadily from a starting  $a^*$  value of approximately 10.3 to 4 over the course of 30 days. The gas flushed sample decreased in this time frame from a starting point of  $a^*$  approximately 11.9 to 9.9 (Haile et al., 2013). Better color stability was seen in the products packed in MAP and with less residual oxygen (75% gas back of 30% CO<sub>2</sub> + 70% N<sub>2</sub>).

## **2.16 Cured meats under Ultraviolet (UV) and visible light**

Both UV and visible light reach the product surface, and while the evidence supports that both contribute to photo oxidation, there is conflicting data regarding which is more damaging. Kampschmidt found that wavelengths of light between 400 and 550 nm provided most of the energy that can be absorbed and used in the reaction (Kampschmidt, 1955). Kampschmidt also observed that cured meat with denatured nitrosomyoglobin had slight differences in absorbed wavelengths from nitrosomyoglobin which included greater absorption of wavelengths beyond 600 nm in cured meat (Kampschmidt, 1955). In a study of canned pasteurized ham (that was sliced and repacked in different packaging materials that varied in OTR and UV barriers (blocking below 360 nm and 250 nm)), Anderson et al. found that UV-light permeability of the packaging material had no effect on color stability (Anderson et al., 1988). This finding was in conflict of an earlier study of steaks that showed UV light in particular was a key contributor of discoloration in fresh and frozen meats (Bertelsen and Skibsted, 1987). Using a continuous wave photolysis technique, Møller et al. exposed purified nitrosylmyoglobin in sealed cuvettes saturated with various gas mixtures to monochromatic light (366 and 436 nm) for time periods between 7 and 14 hours. At oxygen contents of 0.1, 0.5 and 1.0 %, photooxidation of nitrosylmyoglobin was found to have a linear dependent relationship in

both visible (436 nm) and UV (366 nm) spectrums as calculated by  $\Phi_{\text{irr}} = \text{moles of MbFe (II) NO reacted} / \text{moles of photons absorbed by MbFe (II) NO}$  (Møller, Bertelsen and Skibsted, 2002). Exposing nitrosylmyoglobin to 366 nm and 436 nm at 1.5% oxygen found that the quantum yields of photo oxidation were similar at both wavelengths (slightly higher at 366 nm) (Møller, Nannerup and Skibsted, 2005). The quantum yields showed no dependence on CO<sub>2</sub> levels (Møller, Nannerup and Skibsted, 2005). There is evidence to support that in darkness,  $a^*$  values for cured meats in gas flushed or a vacuum remain unchanged (Møller et al., 1999).

## **2.17 Oxygen scavengers and active packaging**

Free oxygen (O<sub>2</sub>) is a major source of color deterioration for most foods (Anderson and Rasmussen, 1992); therefore elimination of oxygen would remove a necessary cause of discoloration of cured meats. Modified Atmosphere Packaging (MAP) and vacuum packaging technologies can improve the shelf life of oxygen sensitive foods by replacing significant O<sub>2</sub> in the package, but these technologies do not always remove O<sub>2</sub> completely (Vermeiren et al., 1999). The use of MAP packaging with cured meat products has created color stability problems when stored under illumination for retail display. Oxygen plays a key role in the discoloration (Møller et al. 2002). Oxygen scavengers are a category within “active packaging” that can minimize the negative effects of oxygen in food deterioration (Kaufman et al., 2000). Active packaging is defined as “an intelligent or smart system that involves interactions between package or package components and food or internal gas atmosphere and complies with consumer demands for high quality, fresh-like, and safe products” (Ozdemir and Floros, 2004). Other active packaging technologies include carbon dioxide emitters/absorbers, moisture absorbers, ethylene absorbers, ethanol emitters, flavor releasing/absorbing systems, time-temperature indicators, and antimicrobial containing films (Ozdemir and Floros, 2004). The commercial potential for O<sub>2</sub> scavengers has been recognized for decades (Labuza and Breene, 1989). Multisorb a leader in the O<sub>2</sub> scavenger industry lists O<sub>2</sub> scavenger benefits as extended shelf life, preventing the growth of aerobic pathogens and spoilage organisms (including mold), and when used with gas flush, an oxygen free interior package. Other benefits include minimizing vitamin oxidation, organoleptic preservation

of foods including color, extended distribution time, and cost savings through reduced waste (Kaufman et al., 2000). Potential risks include anaerobic pathogen growth (such as *Clostridium botulinum*), available moisture to activate the reaction, risk of consuming the sachet, or sachets that leak (while not harmful, could be viewed as product adulteration) (Kaufman et al., 2000). The use of an oxygen scavenger at freezing temperatures ( $-25^{\circ}\text{C}$ ) is possible, but the speed of the reaction is significantly slowed down (Mitsubishi, 2015). Two options to combat the challenge of cold temperatures are using a scavenger designed for low temperatures and storing product for a period of 12 hours at temperatures above freezing to allow for removal of oxygen before freezing (Mitsubishi, 2015). Mitsubishi reports oxygen scavengers in combination with gas flush can be effective, but this is recommended for use with nitrogen flushing only (Mitsubishi, 2015). However the baking industry reports that  $\text{CO}_2$  and  $\text{N}_2$  combinations are affectively executed with MAP and active packaging (Arvanitoyannis, 2012). “Carbon dioxide in the moist environment of an iron-based oxygen absorber will condense and can form ferrous carbonate as some of the iron oxidizes” (T. Powers, personal communication, June 12, 2015). A theory is that the reaction is on the surface of an iron particle forming a barrier that inhibits further oxidation (T. Powers, personal communication, June 12, 2015). This theory is based on the observation that stirring an inactive absorber (which contains some mineral sorbent to carry the moisture necessary for reaction) will reactivate the scavenger (T. Powers, personal communication, June 12, 2015). Multisorb’s FreshPax oxygen absorbers are reported to activate and function normally in atmospheres with up to 50%  $\text{CO}_2$ . “As a result they are ideal for use in MAP packaging applications where the gas mixture is typically 30% ( $\pm 10\%$ )  $\text{CO}_2$ , balance  $\text{N}_2$ ” (T. Powers, personal communication, February 6, 2015).

Performance of scavengers across a variety of foods has proven effective. Oxygen scavengers are both organic and inorganic materials added for the purpose of absorbing oxygen from the environment. They exist in many forms including sachet, films and enzymes (Kaufman et al., 2000). A common  $\text{O}_2$  scavenger uses iron as the active ingredient. Iron reacts with moisture to form iron oxide and hydroxides (Anderson and Rasmussen, 1992). A 65% relative humidity is required to activate. Sodium acts as a catalyst and can reduce the relative humidity required to activate. In considering Oxygen

scavengers as a viable option, it is important to consider the required absorption capacity, absorption rate, and storage temperature (Charles, Sanchez and Gontard, 2006). When using oxygen scavengers, major factors effecting isothermal O<sub>2</sub> absorption kinetics are the humidity level, the O<sub>2</sub> concentration, and the gas composition inside the package (Polyakov and Miltz, 2010). To correctly predict the effect of humidity on the oxygen absorption rate, the porosity, specific surface area (m<sup>2</sup>/kg) and transport properties of the corrosion byproducts from the iron powder need to be considered (Polyakov and Miltz, 2010).

Food varieties including breads, baked goods, nuts, coffee, tea, cured meats and cheeses have all demonstrated improved shelf life when a scavenger is used properly (Alarcon and Hotchkiss, 1993). Bread and cheese have both demonstrated a significant log reduction in mold growth with the application of Freshpax as well as decreased rancidity in peanuts as rated by consumers (Alarcon and Hotchkiss, 1993). Buys concluded that fresh pork when packaged in a 100% CO<sub>2</sub> atmosphere with an oxygen scavenger (Ageless<sup>®</sup> R, Mitsubishi Gas Chemical Company Incorporated, Tokyo Japan) achieved a color-life improvement of 5 days compared to a 100% CO<sub>2</sub> atmosphere without an O<sub>2</sub> scavenger (Buys, 2004). In a study of case ready beef steaks, meat storage failed within 7 days without an O<sub>2</sub> scavenger and permanent discoloration was observed. However with a Freshmax<sup>®</sup> scavenger included (Multisorb Technologies Inc., Buffalo NY), acceptable storage life was increased to as much as 21 days (Limbo et al., 2013). In a study of sliced canned pasteurized ham repackaged with an oxygen scavenger (Ageless<sup>®</sup> SS-50 and GM-50) and a low OTR film (2 cm<sup>3</sup> / [m<sup>2</sup> x day x atm]), discoloration of the ham was found to be completely eliminated in the first 24 hours of display (Anderson and Rasmussen, 1992). In a study of sliced ham in combination with gas flush and vacuum using a low OTR film and an oxygen scavenger (Freshmax<sup>®</sup> Type B & M; Multisorb Technologies Inc., Buffalo NY), Chaiyapechara, Meng and Hotchkiss found lower psychotropic bacteria, yeast, and mold counts, and better color retention (in the form of Hunter L value) when comparing treatments with and without the O<sub>2</sub> scavenger. This study was conducted over 79 days at 10°C under fluorescent light (Chaiyapechara, Meng, and Hotchkiss, 1998).

In a study of sliced cooked ham in Polylactic Acid (PLA) trays, an oxygen scavenger combined with a CO<sub>2</sub> emitter increased shelf life up to 10 days at challenge temperatures of 6-8°C. Even better results were obtained when combined with MAP and a low O<sub>2</sub> level. Measured with a chromameter,  $a^*$  values ranged from 11 to 15 where obtained with a 70% N<sub>2</sub> + 30% CO<sub>2</sub> MAP only, and 100% N<sub>2</sub> with O<sub>2</sub> scavenger and CO<sub>2</sub> emitter; whereas Non-MAP with CO<sub>2</sub> emitter and O<sub>2</sub> scavengers yielded  $a^*$  values from 8 – 9 (Cerioli et al., 2009).

## 2.18 Lipid Oxidation

Lipids in meat products are subject to oxidation and can influence shelf life outcome via causing a rancid product. Cured meat is a food that potentially can have lipid oxidation as a primary mode of failure (Labuza, 1982). Rancidity can be controlled by eliminating oxygen and with the addition of antioxidants like BHA, BHT, and EDTA (Labuza, 1982). Rosemary, black pepper, and ascorbic acid also have antioxidant properties (Martinez et al. 2006). Addition of rosemary and ascorbic acid to fresh pork sausage retarded discoloration in sausages under illumination with a UV filter (but not under standard fluorescent) (Martinez et al., 2006). Phospholipids are more prone to oxidation compare to triglycerides because of greater surface area and unsaturation of fatty acids. Lipid oxidation produces secondary byproducts which are easily measured. One such test is thiobarbituric acid reactive substances (TBARS) test which measures primarily for malonaldehyde (MDA). Malonaldehyde reacts with thiobarbituric acid (TBA) to form a colored compound which can be measure spectrophotometrically (Nielsen, 2010). In a study of fresh beef, alpha and beta-unsaturated aldehydes were found to accelerate oxymyoglobin oxidation (Faustman et al., 1999). In a study of pasteurized ham in MAP, Møller et al. found no significant differences in TBAR values for any of the samples (Møller et al. 2000). In a study of refrigerated sliced cooked ham, the color and lipid oxidative stability were evaluated between light and dark storage. Lipid oxidation was not found to be significantly affected by light (Haile et al., 2013). Haile et al. also commented that TBARS was not an appropriate method to assess lipid oxidation particularly in cured meats, noting that TBAR values lowered after long storage and exposure to light. Haile et al. hypothesized that this may be due to an interaction of MDA

with residual nitrite over time (Haile et al., 2013). It also can be a reaction with amine residues on the proteins (Labuza and Dugan, 1971). Evaluation of cooked cured ham TBARS levels found that nitrite at 100 ppm resulted in a significant reduction of TBARS values at 5 and 14 days refrigeration, suggesting that nitrite has a potential anti-oxidative effect (Han, Yamauchi and Park, 2000). Nitric oxide pigments inhibit lipid oxidation in meat products (Anderson, 1990).

## **2.19 Food Safety – microorganisms and gas flush**

The main defects for meat failing microbiological shelf life are off odors and flavors, but meat discoloration and gas production can also occur (Borch, Kant-Muermans and Blixt, 1996). A meat discoloration phenomenon associated with microorganisms involves the bacterial production of hydrogen sulfide and greening of the product (Borch, Kant-Muermans and Blixt, 1996). *Lactobacillus sake* is capable of forming hydrogen sulfide, but only when glucose and oxygen availability is low (Egan, et al 1989). Meat discoloration is not always an indication of unsafe product. In a study of sliced pasteurized ham, total plate counts between discolored and color stable product showed no differences (Møller et al., 1999).

There is not a significant amount of microbial data for gas flush products with multiple components like a sandwich represents. Data is available for meats, starches and cheese as separate components. The predominant bacteria associated with spoilage of refrigerated beef and pork includes *Brochothrix thermosphacta*, *Carnobacterium* spp. *Enterobacteriaceae*, *Lactobacillus* spp. *Leuconostoc* spp. *Pseudomonas* spp. and *Shewanella putrefaciens* (Borch, Kant-Muermans and Blixt, 1996). Fresh pork loin packaged in a variety of CO<sub>2</sub> & CO environments resulted in a dominate lactic acid bacteria environment, where *Salmonella* was not detected, showing a 2.5 to 3.5 log unit growth (Viana, Gomide, and Vanetti, 2005).

Bacteria associated with refrigerated meat products are *B. thermosphacta*, *Carnobacterium* spp. *Lactobacillus* spp. *Leuconostoc* spp. and *Weissella* spp. (Borch, Kant-Muermans and Blixt, 1996). Cooked cured meats often are stored under vacuum and MAP (Metaxopoulos, Mataragas and Drosinos, 2002). Significant literature exists demonstrating gas flushed products promotes a favorable environment for *Lactobacillus*

and suppresses other spoilage organisms, but growth rates are slowed under refrigeration (Farber and Dodds, 1995; Borsch, Kant-Muermans and Blixt, 1996). As lactic acid bacteria grow, production of antimicrobial substances (likely bacteriocins) inhibits the growth of other spoilage organism (Metaxopoulos, Mataragas and Drosinos, 2002). Inhibition of other organisms by lactic acid bacteria is attributed to lactate and hydrogen peroxide production (though not likely if the environment is completely oxygen free) (Metaxopoulos, Mataragas and Drosinos, 2002).

While *Lactobacillus* microflora is dominant, many pathogenic organisms are less affected by MAP (Farber and Cai 1996). *Lactobacillus* growth under refrigeration is slowed, leaving the potential for extended growth of a pathogen in the event of contamination. This makes refrigerated psychotropic pathogens of particular concern, including *Listeria monocytogenes*, nonproteolytic *Clostridium botulinum*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* (Farber and Dodds 1995).

For *Listeria*, the best line of defense is Good Manufacturing Practices (GMP) and sanitation programs. As *Listeria* is not a native organism to cured meats, cheese or bread, preventing it from getting in the product prevents concern. It is noteworthy that high CO<sub>2</sub> levels have shown in predictive models, a decrease in the lag time and generation time of *Listeria monocytogenes* (Farber, Cai and Ross, 1996). Using a brain heart infusion broth, Farber, et al. showed that over a 30 day period at a pH of 5.5 and temp of 4°C, *Listeria* was unable to grow in the presence of  $\geq 50\%$  CO<sub>2</sub>. The use of lactate, diacetate and irradiation also is effective in controlling *Listeria* growth in RTE meats in vacuum pack (Knight et al., 2007). However, the addition of lactate in cured meat has also been shown to reduce residual nitrite in the product (McClure et al., 2011). This is good from the standpoint that lactate has been hypothesized to produce NADH which can reduce metmyoglobin to deoxymyoglobin (improving color), but is not as favorable for inhibition for potential *C. botulinum* as nitrite is considered a strong antimicrobial to control *Clostridium botulinum* spores from outgrowth (Shahidi and Pegg, 1992). When considering a low level or elimination of O<sub>2</sub> from the environment, careful consideration needs to be given to anaerobic microorganisms like *Clostridium botulinum*. Development of botulism is the result of ingesting the toxins produced by strains of the organism. Non-proteolytic strain growth (type E and some type B & F) is inhibited at



pH<5, 5% NaCl and temperatures below 3.3°C (Hutchinson, 1992). Cured meats rely on reduced heat treatments and salt & nitrite to inhibit growth (Hutchinson, 1992).

Using steamed rice in MAP (0 and 15% oxygen (with 5% CO<sub>2</sub> and 5% N<sub>2</sub>) challenge studies using a mixture of *Clostridium botulinum* (five strains of type A and five strains of type B) found no neurotoxins for 24 weeks at 15% oxygen conditions. However, botulinum neurotoxin was found in one of three samples after 12 weeks and in one of two samples at 24 weeks in 0% oxygen and 30°C (Kasai et al., 2005). Ascorbate and erythorbate have also been found to help control *Clostridium botulinum* but there is conflicting evidence as to the validity of this work (Varnam and Sutherland, 1995). Dry-cured ham over the course of 8 weeks in vacuum, 100% N<sub>2</sub>, 20% CO<sub>2</sub> + 80% N<sub>2</sub> found no significant difference in microbial quality. The dominant organisms were mesophilic aerobic colonies, lactic acid bacteria, yeast and mold. *L. monocytogenes*, *Campylobacter jejuni*, & *Salmonella* were undetected (García-Esteban, Ansorena and Astiasaran 2004). Dry cured Iberian ham sliced stored under a variety of gas compositions demonstrated no safety issues regarding microbial quality. No significant differences were detected for *Enterobacteriaceae*, *Escherichia coli*, yeast & mold throughout the 120 day shelf life. In addition, *Campylobacter* sp., *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus* and *Vibrio* sp. were analyzed and undetected (Parra et al., 2010). Growth in canned pasteurized ham was found to be dominated by lactic acid bacteria, and *Brochothrix thermosphacta* wasn't detected (Anderson, 1990). Dry cured beef product in vacuum, 20% CO<sub>2</sub> + 80% N<sub>2</sub> and 80% CO<sub>2</sub> + 20% N<sub>2</sub> at 6°C found values from day 0 to 210 to not change significantly. *Pseudomonas* numbers were significantly inhibited and the typical microflora consisting of Lactic acid bacteria, yeast and mold, and *Micrococcaceae* were unchanged (Rubio et al., 2007).

While further research is needed, the dominant *Lactobacillus* environment and the presence of nitrite in the product provides some measures to prevent *Clostridium botulinum*.

E.A. Sween company routinely tests products for microorganisms. Non-pathogenic specific flora is not tested in ham and cheese sandwiches. The product routinely starts out with low counts, (<10 cfu/g). These results have been consistently duplicated (see Table

2.4). The microflora is varied (heterogeneous) and is based on the processing environments related to the individual product post cook going into the assembly. Typically you could find *Bacillus* spp., *Micrococci*, *Flavobacterium*, *Pseudomonas*, *Enterococcus*, *Lactobacillus*, yeast and lactic acid bacteria (*Lactococcus*, *Leuconostoc*, *Pediococcus*) (Jay 1996). After storage, depending on storage conditions (temperature, packaging/O<sup>2</sup> Perm/non O<sup>2</sup> perm, MAP, non-MAP, vacuum, etc.) the predominant bacteria is likely be lactobacillus or lactic acid bacteria (Jay 1996).

Ultimately, the shelf life failure of a gas flushed sandwich due to microorganisms will be due to off flavors, odors and gas production (Borsch, Kant – Muermans and Blixt, 1996). It is noteworthy that a lactic acid bacteria count in the range of 10<sup>6</sup> – 10<sup>7</sup> pass organoleptic screening for Deli Express with no off flavors or odors detected. The typical mode of failure of a 30-day refrigerated sandwich is excess gas production causing “puffy packs”.

While further research is needed, the dominant *Lactobacillus* environment, the presence of nitrite and salt, the bacteriostatic effect of CO<sub>2</sub> provides some measures to prevent facultative or anaerobic psychotropic pathogens as a concern. It is incumbent on the manufacturers of modified atmosphere products to verify the shelf life and safety of the products (Sofos, 1993). Methods of verification include challenge studies with pathogens to verify growth conditions. Challenge studies for the Ham and Cheese sandwich has demonstrated no *Listeria* growth (Table 2.5).

**Table 2.4** Microbial results for ham & cheese sandwich with corresponding Carbon Dioxide and Oxygen.

Code 951 Smoked Ham and cheese					Code 772 Smoked Ham and cheese				
test date		8/14/2012			test date		8/6/2014		
Day	CO2 %	O2 %	Lactics (cfu)	Yeast & Mold (cfu)	Day	CO2 %	O2 %	Lactics (cfu)	Yeast & Mold (cfu)
0	17.5	0.412	<100	<100	0	20.6	14 ppm	<10	<10
0	17.3	0.434	<100	<100	1	20	0.153	<10	<10
7	16.8	0.619	<100	<100	14	19.7	20 ppm	<100	<10
7	17.2	0.257	<100	<100	14	20.2	18 ppm	<100	<10
14	16.3	0.555	<100	<100	30	19.4	42 ppm	<100	<10
14	16.2	0.306	<100	<100	30	19.7	38 ppm	<100	<10
28	16.6	0.236	<100	<100	37	19.2	45 ppm	<100	<10
28	17.7	0.228	<100	<100	37	19.4	40 ppm	<100	<10

**Table 2.5** *Clostridium botulinum* and other Challenge Studies for Deli Express® products


Clostridium botulinum and other Challenge Studies for Deli Express Products to Date													
Date	Name (of Sand.)	Inoculated C.bot	Organisms/Initial E.coli O157:H7	Levels (CFU/g) Salmonella	S. aureus	Listeria	Uninoculated Organisms Lactics (MRS)	Gas Analysis CO2/O2	Storage Temporaries (Degrees C)	Length of Time	Freq. of Sampling	Result	
Apr-95	Ham and Cheese Wedge	Log 4	N/A	N/A	N/A	N/A	yes	yes	4/10/16/22/37	28d	2wks	No Growth	
Dec-98	Ham and Cheese Sand.	log 2	N/A	7.30E+06	4.00E+07	N/A	yes	yes	4/10/30	120d	2wks	No Growth	
Apr-01	Ham and Cheese 24	log 2	N/A	1.70E+03	3.50E+02	N/A	yes	yes	7/12/30	56d	7d	No Growth	

### **3 Methods and materials**

#### **3.1 Sandwich ingredients**


Unless otherwise noted, the ham evaluated in all studies is a water added cured and cooked smoked ham produced by ®. The average age of the hogs utilized is 6-7 months with an average live weight of 275 pounds. The muscles utilized are insides (semimembranosus and adductor) with a ground portion (from the shank) added. The formula also includes the gracilis muscle which typically has more pigment. There is also an area by the cap and a second area sometimes referred to as the corner/kernel/tip (artery corner) utilized, that also has a slightly deeper red color. Inside muscle also contains an area referred to as the red eye. Red eye is the part of the inside muscle that is nearest the femur bone. Muscles are thoroughly macerated with the ground portion passing through a 5/64" (hole diameter) plate and then mixed with curing brine containing 0.01838 kg of sodium nitrite per 100 kg of meat. 29 kg of curing brine (which includes water, salt, phosphate, and nitrite) is added per 100 kg of meat for a total weight of 129 kg. Mixtures are tumbled for 1 hour and pumped into a smoked plastic casing for 4.5-6 hours cooking time. The plastic casing is made of a proprietary combination of food grade cellulose paper, polyethylene and nylon film with O<sub>2</sub> permeability of 3 cc/m<sup>2</sup>/24hr/atm and water vapor permeability of 10.7 g/m<sup>2</sup>/24hr (Viscase®). After heating, the cooked cured pork is placed in a blast cooler for 4-6 hours until achieving < 40° F. The initial nitrite level is 184 ppm (parts per million), with an estimated residual nitrite level of 20 ppm as reported by the supplier (D.Witte, personal communication, May 15, 2015 ). The FDA limits for sodium nitrite in meat curing is “not more than 200 parts per million in the finished meat product, and the amount of sodium nitrate to not more than 500 parts per million in the finished meat product (CFR, 2015). The percent fat 2.77%. The initial appearance of the ham is not uniform with several shades of pink and red, and some visual marbling (Table 3.1). The product is processed and packaged in a plastic casing with an expected shelf life of 120 days @ 28-32°F. The age of the ham log at the point of slicing for placement on the sandwich is 7 to 60 days.

**Table 3.1** Ham product characteristics prior to sandwich processing (As reported by the manufacturer)

<b>Flavor:</b>	Ham flavor, some saltiness & some smoke flavor
<b>Texture:</b>	Some bite / resistance (like a full muscle)
<b>Product Appearance:</b>	<p>Small amount of marbling, and some dark red spots, should not look blended or emulsified</p> 
<b>Water Activity (<math>a_w</math>):</b>	0.95-0.97
<b>% water added:</b>	17% PFF (Protein Fat Free is the meat protein content expressed as a percentage of the non-fat portion of the finished product)
<b>pH:</b>	6.0 to 6.5
<b>Ingredients:</b>	A Portion of Ground Ham Shank and Ham Added [Cured with Water, Salt, Contains 2% or less of Modified Food Starch, Corn Syrup, Dextrose, Potassium Lactate, Sodium Lactate, Sugar, Sodium Phosphates, Sodium Diacetate, Sodium Erythorbate, Sodium Nitrite].
<b>Manufacturer:</b>	John Morrell®, Sioux Falls, SD


Two slices of a 7/16” thick white bread with an approximate total weight of 2 ounces was used per sandwich (Product specification in Table 3.2).

**Table 3.2** White bread product characteristics prior to sandwich processing (As reported by the manufacturer)

<b>Flavor:</b>	Typical white bread – bland
<b>Texture:</b>	Soft bite, more airy than dense
<b>Product Appearance:</b>	Off white, textured surface 
<b>Water Activity (<math>a_w</math>):</b>	0.91 to 0.95
<b>pH:</b>	4.5 to 5.5
<b>Ingredients:</b>	Unbleached Wheat Flour, Water, High Fructose Corn Syrup, Yeast, Liquid Soy Oil, Salt, Whey Solids, Yeast Nutrients (Monocalcium Phosphate, Calcium Sulfate, Ammonium Sulfate, Potassium Iodate), Monoglycerides, Dough Conditioners (Sodium Stearoyl Lactylate, Calcium Peroxide), Malted Barley Flour, Calcium Propionate (To Retard Spoilage), Ferrous Sulfate (Iron), Niacin, Thiamine Hydrochloride (Vitamin B1), Riboflavin (Vitamin B2), Folic Acid.
<b>Manufacturer:</b>	Pan O Gold Bakery, St. Cloud, MN

One slice of pasteurized process American cheese with an approximate weight of 0.4 oz. was used per sandwich (Product specification in Table 3.3).

**Table 3.3** Processed American cheese characteristics prior to sandwich processing (As reported by the manufacturer).

<b>Flavor:</b>	Creamy, salty, processed and mild American cheese flavor
<b>Texture:</b>	Smooth, soft
<b>Product Appearance:</b>	Orange color, smooth surface 
<b>Water Activity (<math>a_w</math>):</b>	0.95 target
<b>Moisture:</b>	40% maximum
<b>pH:</b>	6.0 Maximum
<b>Ingredients:</b>	American Cheese (Pasteurized Milk, Cheese Cultures, Salt, Enzymes), Water, Cream, Sodium Phosphate (Emulsifier), Sorbic Acid (Preservative).
<b>Manufacturer:</b>	Schreiber Foods®, Carthage, MO

### 3.2 Oxygen scavenger sachets

Multisorb Freshpax® oxygen absorbing packets were selected to absorb oxygen quickly. The packets are approximately 1” x 1.75” (Figure 3.1) and are placed in the package for direct contact with the head space (the packet will also be in direct contact with the food).



**Figure 3.1** Appearance of Multisorb scavenger

Type D scavengers were designed to be optimal for dry foods, but the type B application (designed for moist foods) has several hours activation time during which it adsorbs moisture from the atmosphere in the package before it begins to absorb oxygen. Given the product undergoes a slow freeze soon after assembly, (placed in a freezer operating at approximately -15°C) the goal in selecting the type D was to optimize the amount of oxygen scavenged in the first 24 hours in the freezer before a significant freeze down is achieved. The type D design is patented, but the active ingredient is iron, and a salt and moisture source is included. Because the packet has a moisture source, it is immediately active and begins to absorb oxygen as soon as it diffuses into the packet (which requires the packets be vacuum packed prior to adding to the sandwich) (T. Powers, personal communication, June 12, 2015). Requirements for optimal use are an adequate oxygen barrier film ( $<1$  cc of oxygen / 100 in<sup>2</sup>/24 hours), hermetic seals (3/8" wide), and free circulation around the product.

D-50 cc has a more aggressive capacity than required by calculation (1 cc of oxygen was estimated for removal in the ham & cheese sandwich package— see Table 3.4), but was selected to insure rapid absorption.

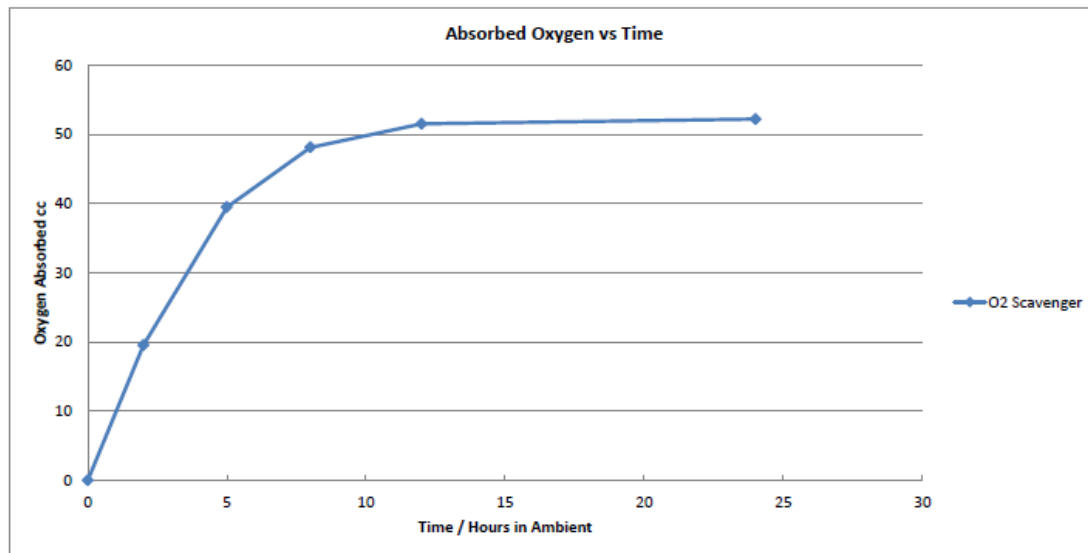


**Table 3.4** Calculation for amount of oxygen estimated to be removed from a wedge shaped MAP sandwich package (Two packages are place together to form a cube. The dimension of a cube is 3.25'' x 4.75'' x 4.5'')

1 cubic centimeter = 0.06102 cubic inches	
Cubic inches for the cube	93.75
cc for the cube	1536.29
cc for 1/2 the cube	768.14
estimated void space in the package (defined as open areas between bread, meat & cheese)	25.00%
headspace (amount of space between the sandwich and the package)	0.00%
cc of void space	192.04
oxygen in the package	0.50%
cc of oxygen in the package	0.9602

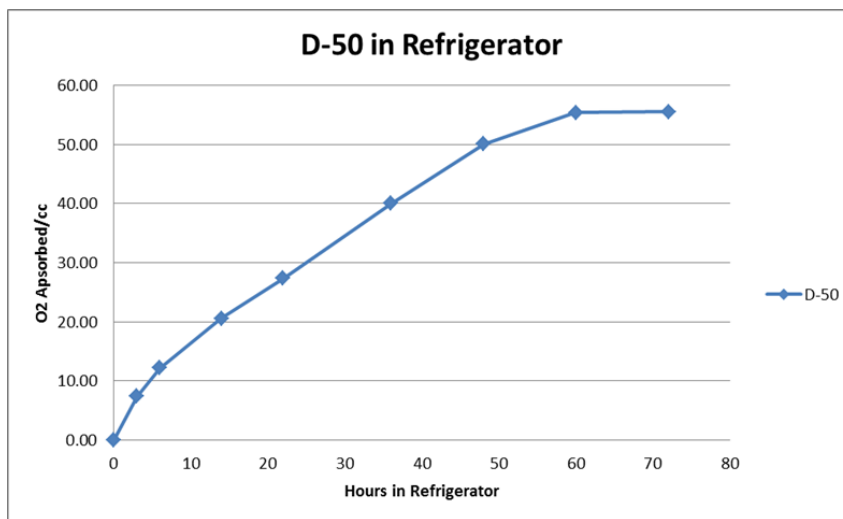


At room temperature, D-50 is capable of absorbing 50 cc of oxygen in approximately 9 hours. (Figure 3.2)



**Figure 3.2** Multisorb D-50 absorption rate at room temperature (empty package) (D. Elliason, personal communication, June 16, 2014)

At refrigerated temperatures, D-50 is capable of absorbing 50 cc of oxygen in approximately 49 hours (Figure 3.3).



**Figure 3.3** Multisorb D-50 absorption curve in a refrigerator (empty packages) (D. Elliason, personal communication, June 16, 2014)

The sandwich once assembled will spend no more than ½ hour at temperatures ranging 5-10°C. It is then placed in frozen storage -12 to -18°C for up to 3 months. It is assumed

that once the sandwich and O<sub>2</sub> scavenger reaches a frozen state, there is little activity; however in the 24 hours prior to reach this state, the scavenger is working. For a spooled D-50 scavenger the price is approximately \$27.00 - \$32.00 / 1000 (\$0.027 - \$0.032 per package). The product specification for D-50 is listed in Appendix F.8.

A D-30 scavenger was used in tests 4 and 5. The product specification for D-30 is listed in Figure 3.5. The cost of a spooled D-30 scavenger is approximately \$25.00 (\$0.025 per package). Product is also available as individual sachets, however for placement of the packets, there is recommended equipment (Figure 3.4) that provides protection from premature scavenging, and labor savings on placement. The capital cost of the placement equipment is approximately \$65,000 (D. Elliason, personal communication, June 12, 2015).



**Figure 3.4** Sachet placement equipment (Multisorb, 2014) [www.multisorb.com](http://www.multisorb.com).

### 3.3 Top non-forming packaging film – clear / transparent

Non-forming film is film that is not stretched or formed during the packaging process.

Forming film is stretched during the packaging process to form a pouch. The minimum acceptable thickness of forming film is 1 millimeter (mil).

The top non-forming film used is a lamination of a 50 gauge polyethylene terephthalate (PET) / 200 gauge peelable linear low density polyethylene (LLDPE) co-extrusion (two ply lamination) that is 2.6 millimeter thick. Film properties include a WVTR (Water Vapor Transfer Rate) of 0.3 gm/100 in<sup>2</sup>/24 hour, oxygen Permeation Rate of <0.5 cc/100 in<sup>2</sup>/24 hour and an oxygen barrier layer composition of ethylene vinyl alcohol (EVOH). The packaging appearance is transparent. The film is produced by Belmark in De Pere, WI.

### **3.4 Bottom forming packaging film – black and clear**

The black pigmented bottom forming film used is a proprietary coextruded film with EVOH as the active barrier to oxygen, polyester sealants & nylon structural layers. The starting thickness is 8 millimeter (mil), with a minimal thickness of 1 mil after forming. Barrier properties include oxygen <0.30 cc per 100 in<sup>2</sup> per 24 hours at 73°F and 0% RH (Relative humidity), WVTR <0.5 grams H<sub>2</sub>O per 100 in<sup>2</sup> per 24 hours at 100°F and 90% RH. The packaging appearance is black. The film is produced by Bemis® Curwood in Osh Kosh, WI.

The clear bottom forming film used was CURLON® (Grade 9581-AA) manufactured by Curwood® (Osh Kosh, WI). This film is a flexible, formable web for protective packaging of products which are suitable for vacuum and gas applications where low O<sub>2</sub> levels are required. Recommended for high speed packaging applications where package clarity, outside package C.O.F., uniform formed distribution, and package tightness are critical package criteria (Appendix L.1). The oxygen transmission rate is O<sub>2</sub> < 0.30 CC per 100 in<sup>2</sup> per 24 Hrs at 73°F & 0% RH. The Moisture Vapor Transmission Rate (MVTR) is MVTR < 0.5 gm H<sub>2</sub>O per 100 in<sup>2</sup> per 24 Hours at 100°F & 90% RH.

### **3.5 UV blocking films**

Several combinations of ultraviolet (UV) blocking films were explored in test 5. This includes the structural combinations of 1) PET/adhesive/UV Sealant #1, 2) UV PET/adhesive/UV PET/adhesive/ UV sealant r #1, and 3) PET/adhesive/UV sealant #2. The adhesive used is a polyester polyurethane solvent less adhesive system with EVOH located as a co-extrusion layer in the sealant. The UV PET blocks from 350nm to

400nm, with a continuous reduction on the UV light blocking that is better than a regular non-UV PET film. At 360nm UV PET blocks 87% while non-UV PET blocks only 18% (5 times better). At 380nm UV PET blocks 42% while non-UV PET blocks only 17% (2.5 times better). At 400nm both UV PET and non-UV PET block 16% (J.Vandeloo, Belmark, personal communication November 25, 2014).

Using a UV additive in the sealant (at the intended percentage) blocks about 98% UV at 300 nm, 90% at 375 nm and almost 60% at 400 nm (S. Utecht, Belmark, personal communication, August 03, 2012). The addition of the additive in a 2 mil sealant film potentially increases the haze by a factor of 2x. There is notable cost associated with this technology, especially for a food safe option. The cost is approximately 20% higher than the control (adds approximately \$.002 per sandwich) (J.Vandeloo, Belmark, personal communication November 25, 2014).

UV sealant #1 and UV sealant #2 are different UV technologies. UV sealant #1 is using UV absorbing technology. This technology “screens” UV light from penetrating to the packaging contents. UV sealant #2 is using UV blocking technology. The additive functions by allowing visible light to pass through and preferentially scatters the light in the UV spectrum. It is especially effective in blocking UV transmission in the 250-350nm range, blocking about 80% of UV in this range. (S. Utecht, Belmark, personal communication, August 03, 2012) The packaging appearance is primarily transparent. The film is produced by Belmark in De Pere, WI.

### **3.6 Ferrous based non forming scavenging film**

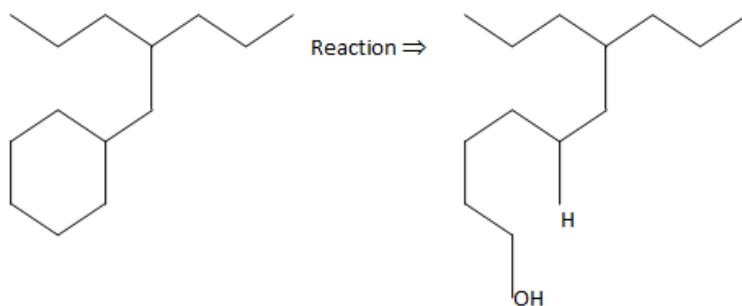
Winpak® combines passive and active barrier technologies in a polyester lamination with a high barrier EVOH and linear low density polyethylene co-extrusion sealant. While the passive barrier EVOH is designed to stop the ingress of oxygen, the active barrier removes intra-package oxygen using a chemical absorption process. The chemical absorption process converts the ferrous iron powder buried in the sealant film and any free oxygen in the package into a stable ferric oxide. In this system there is no generation of byproducts that may affect the organoleptic properties of the food. The packaging appearance is translucent, with a strong gray tint. The film is produced by Winpak® Ltd. in Manitoba, Canada. The cost increase when comparing to a similar structure without

the oxygen scavenger component is approximately 50% greater with the scavenger component added (R. Klips, personal communication, August 10, 2015).

### 3.7 Non-ferrous based non-forming scavenging film and complimentary forming film

The non-ferrous based non forming film is a multilayer coextruded film that incorporates both oxygen barrier and oxygen scavenging layers. (Appendix H.8) The film comes with a biaxial oriented PET outer layer. Based on a system of proprietary technologies, this polymer based method reduces oxygen levels in MAP applications. Scavenging begins when a patented UV light triggering unit (installed on the packaging line) activates the film. The scavenging polymer is incorporated into the package and is invisible to the consumer. The mechanism of scavenging is accomplished when ethylene methyl acrylate cyclohexene methanol is exposed to UV light. (Figure 3.5)

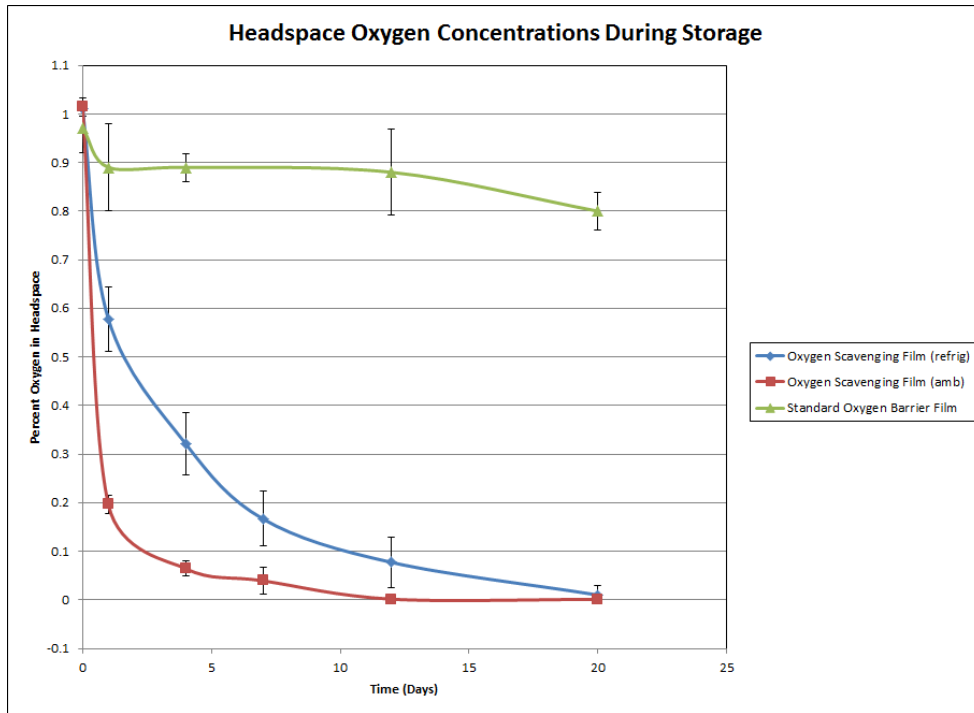
Oxygen Scavenging Reaction:



**Figure 3.5** Non-ferrous based scavenging reaction (S. Beckwith, personal communication, November 22, 2013)

The ring is able to oxidize with sufficient UV energy with the presence of a catalyst (cobalt). Optimal performing temperature range is 3.3 – 21°C. (S. Beckwith, personal communication, February 25, 2015) The film is produced by Cryovac® in Duncan, SC. The non-form film is used in conjuncture with a forming web that contains an oxygen barrier layer that has some oxygen scavenging polymer blended into the barrier resin for

an additional level of oxygen ingress protection. The bottom forming film does not scavenge headspace oxygen and does not require UV activation because it is extruded in the active form. Figure 3.6 shows the oxygen scavenging capability of the film for an extended shelf life pasta product.



**Figure 3.6** Changes in headspace oxygen for fresh pasta during storage use the non-ferrous based scavenging film (S.Beckwith, personal communication, November 22, 2013)

### 3.8 pH measurement method

The pH was measure using a pHTestr 10 BNC (Osprey Scientific, Edmonton, Alberta Canada). Each sandwich component was removed from the package and separated, 10 grams were weighed out, and diluted in 100 ml of deionized water, and placed in a Masticator (IUL instruments, Barcelona Spain). Liquid was filtered to remove solid particles. The pH was measured in the fluid removed. A single pH reading was taken from bread, ham and cheese on day 4, and at day 30. The sandwiches measured at day 4 and 30 were from the same production batch.

### 3.9 pH values of the sandwich components

Each sandwich component has a unique individual starting value pH range as reported by the manufacturers (Tables 3.1 – 3.3). As the shelf life precedes, the pH of each component changes, as the heterogeneous components equilibrate with direct contact with each other, the microflora grows in the MAP environment (*Lactobacillus*), and Carbon Dioxide dissolves into the components forming carbonic acid. As a result of these dynamics, the bread pH increases over time, while the ham and cheese decrease (Table 3.5). the direction of change is due to the same factors as water activity. The bread starts out at the lowest pH and increases while the other two components decrease and all come close to each other.

**Table 3.5** pH readings by component at day 4 and day 30 of refrigeration, each component received a single measurement at each day. The sandwich used at day 4 and 30 were from the same production batch

Component	starting pH reported by supplier	Measured pH day 4	Measured pH day 30
Bread	4.5 - 5.5	5.7	5.82
Meat	6.0 - 6.5	6.21	5.87
Cheese	6.0 maximum	6.1	5.86

A repeat test was set up to measure the pH in triplicate at days 1, 7, 14 and 25. The results of this evaluation demonstrate that pH does change over time (Ham starting average pH of 6 which decrease to 5.89 by day 14) , however there is variability from day to day which provides another changing condition that may explain variation from package to package (Table 3.6).

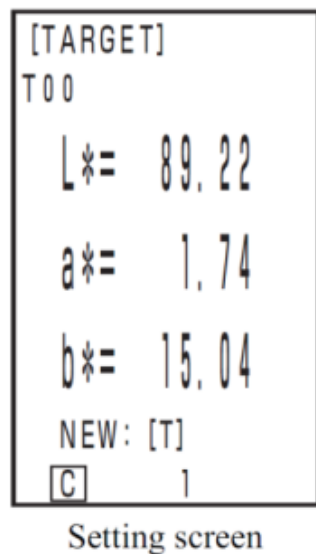


**Table 3.6** follow up pH check of each sandwich component part at multiple points of refrigerated storage

6/17/2015	Day 1:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	5.88	5.96	5.42
	<b>2</b>	6.07	6.03	5.32
	<b>3</b>	5.96	6.01	5.40
	average	5.97	6.00	5.38
	min	5.88	5.96	5.32
	max	6.07	6.03	5.42
6/25/2015	Day 7:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	6.19	6.00	5.71
	<b>2</b>	6.21	5.94	5.72
	<b>3</b>	6.17	5.97	5.74
	average	6.19	5.97	5.72
	min	6.17	5.94	5.71
	max	6.21	6.00	5.74
7/1/2015	Day 14:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	6.02	5.87	5.60
	<b>2</b>	5.98	5.90	5.65
	<b>3</b>	6.06	5.91	5.70
	average	6.01	5.89	5.65
	min	5.98	5.87	5.65
	max	6.06	5.91	5.70
7/15/2015	Day 28:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	6.17	6.03	5.73
	<b>2</b>	6.18	5.98	5.81
	<b>3</b>	6.15	5.95	5.82
	average	6.17	5.99	5.79
	min	6.15	5.95	5.73
	max	6.18	6.03	5.82

### 3.10 Color determination

In accordance with ASMA recommendations for modified atmosphere packages, multiple sub-samples were prepared from an original sample production run. Ham color was measured after each sealed packaged was analyzed for the atmosphere head space (Hunt et al., 2012). Because obtaining O<sub>2</sub> measurements from the head space of the package breaks the MAP seal (accomplished by piercing with a needle to obtain a sample of the gas for analysis), all samples were removed from the package for color measurement and visual inspection and discarded. For all tests, one sample of each test condition was removed from the refrigerator and measured in three locations on the ham surface on designated days throughout the time study. Direct comparisons were made among test conditions from similar locations in the cooler. All products were removed from the package and analyzed for CIE  $L^*$ ,  $a^*$ , and  $b^*$  for Illuminant C using an aperture of 50 mm and the standard observing angle of 2° using a Konica Minolta Chroma Meter CR-410. (Minolta, Osaka, Japan) Prior to each session, the chroma meter was calibrated to the white calibration screen (Figure 3.7).

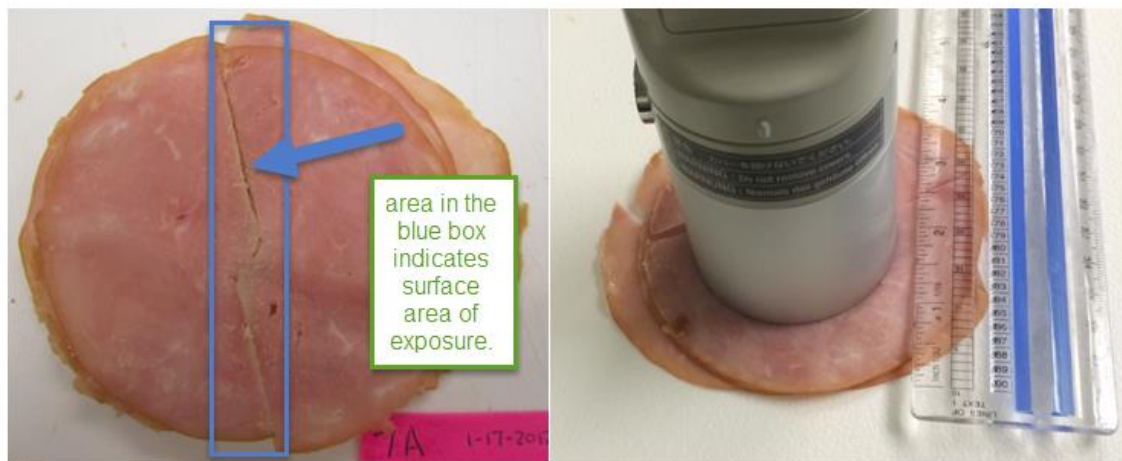


**Figure 3.7** Calibration targets for chromameter

Seven slices of ham were stacked (approximately 10 mm thick), and placed on a white cutting board for color measurements. This represents the typical thickness of ham on a

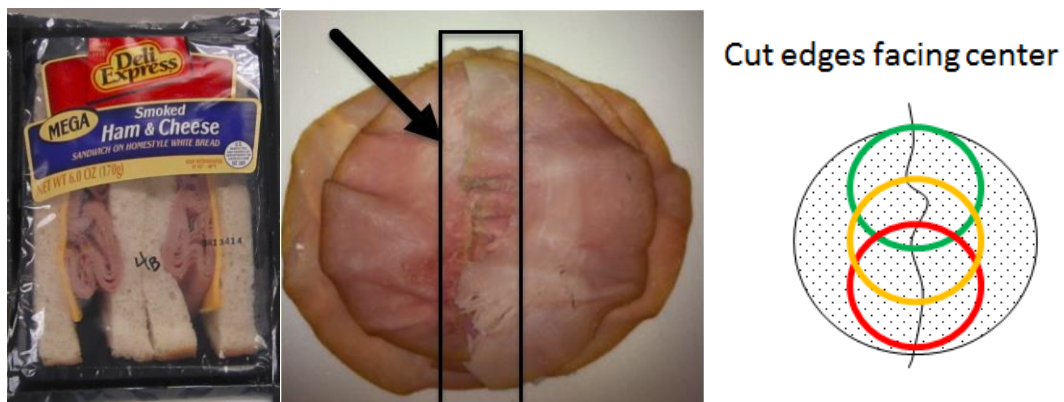
Deli Express sandwich. ASMA suggests that a thickness of 12 to 15 mm should be sufficient to absorb non-reflected light, or if less than, that a white cutting board should be used to prevent light from passing through (Hunt et al., 2012).

The diameter of the ham is approximately 4". The diameter of the Chroma meter lens is 1.9685". Using the area of a circle ( $a = \pi * R^2$ ), the lens captures approximately 24% of the ham slice surface area. Of the area measured, only 8% of the surface area contains surface exposed to light and  $O_2$  (approximately 1/8" wide by 1.9685", Figure 3.8 the area within the blue box). This measurement method is not ideal given the limited amount of exposed ham surface area to light and  $O_2$  being captured in the color measurement, however it is important to evaluate the retail appearance of the product as the consumer sees it, and use available equipment at EAS.



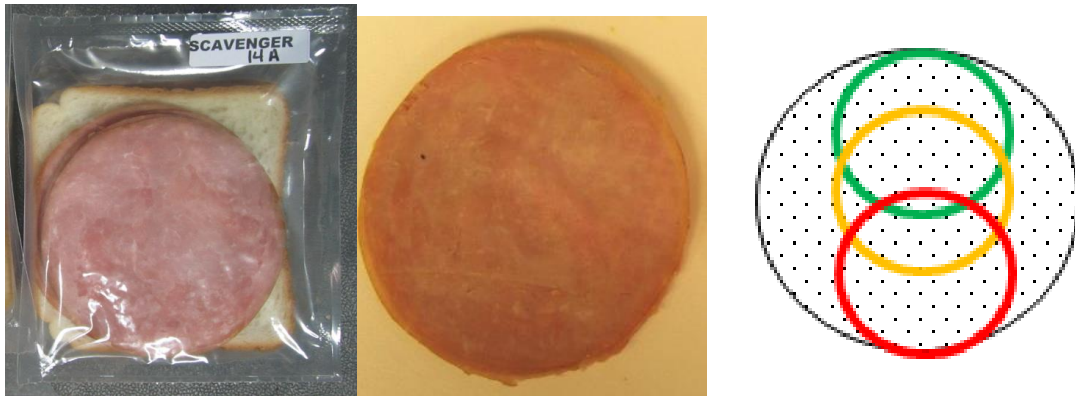
**Figure 3.8** Chromameter measurement area on ham surface

A white cutting board was placed under each sample per ASMA recommendations. Three measurements were obtained on the face slice of each test condition sample by directly pressing the lens on the meat surface. In tests 1-5, the bunched meat was flattened out and reconstructed back into a full flat slice. This is referred to as method 1 (Figure 3.9).



**Figure 3.9** Method 1 – used in tests 1-5. A) Sandwich was stored on the refrigerator shelf as the consumer would see it B) Bunched meat was reconstructed back into a full flat slice, by flattening out the “bunched” ham and with the ham exposed to light represented through the middle of the slice C) Sample measured three times in the middle section (with green, yellow, and red circles representing the chroma meter measuring head measurement locations after reconstructing the slices)

Tests 6 – 11 used ham placed flat on top of the bread and cheese for full surface area exposure to light. This is referred to as method 2 (Figure 3.10).



**Figure 3.10** Method 2 - measurement method tests 6-10 A) Sandwich was packaged with bread on the bottom, followed by cheese, with ham placed flat for full exposure to the headspace oxygen and light. B) appearance of flat slice prior to color measurement C) Sample measured three times in the middle (with green, yellow, and red circles representing the chromameter measuring head measurement locations after reconstructing the facing)

The three measurements were averaged together to represent a single CIE  $L^*a^*b^*$  value for the sample. With limited cooler space with close proximity to the light source, only one sandwich per test condition was measured each designated shelf life day. The studies ranged from two to five test conditions per test (one condition always serving as the control). Using the close door model EAS provides to the industry as the primary cooler, the light source is located on one side with a total of eight spaces available nearest the light source for direct comparison among test conditions. Cooler availability varied throughout the study, with a maximum of five coolers for the final studies. The majority of the studies were conducted from three coolers. With three coolers with eight locations nearest the light source, a total of twenty-four samples were available for direct comparison. The goal was to obtain color measurements on a minimum of five day intervals throughout the shelf life for each test condition, which often limited the number of replicates per test condition per color measurement day to one.

Care was taken to avoid pillowing of the meat (forming a curved surface under the lens (which can affect the color reading)). (Hunt et al., 2012) All samples images were captured using a Canon Power Shot SD1400 IS Digital Elph, 14.1 Mega Pixels, Canon Zoom Lens 4x15, 5.0-20.0mm 1:2.8-5.9, Canon PC1472 4.3 V camera. These pictures

are found in Appendix A – K. Visual observations were made by 2 people prior to photographing.

Method 1 (test 1-5) involved starting with the sandwich the way consumers see it on the store shelf, placing the two sandwich halves back together, discarding the bread and cheese, and flattening the bunched meat so that the exposed portion of the ham to light and headspace oxygen is in the middle of the slice where the two halves meet. By reconstructing “bunched” meat and flattening out, this provided a flat measurable surface for the Chromameter to measure. (Figure 3.9) Method 2 was implemented for tests 6-11. In this method, the same amount of ham, cheese and bread were used as in method 1, but instead of bunching the ham and cutting into the wedge shape, the meat was left flat and placed on top of the cheese and bread to obtain greater surface area exposure to light and headspace oxygen (Figure 3.10). Three measurements were taken of the ham (top, middle, and bottom) and averaged to represent a single  $L^*a^*b^*$  measurement per sample.

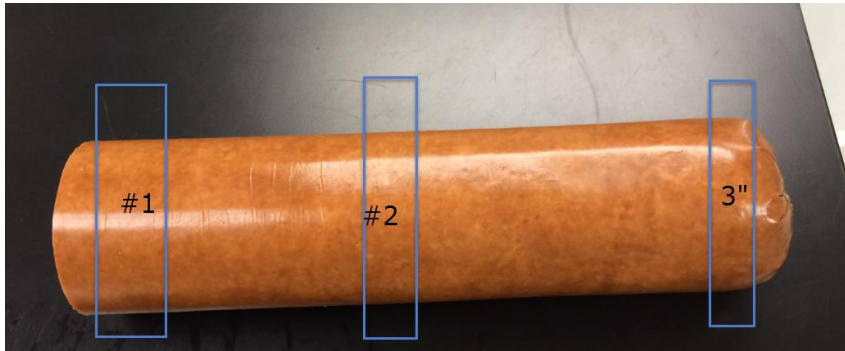
### 3.11 Initial ham CIE $L^*a^*b^*$ values

The CIE  $L^*a^*b^*$  values for the ham prior to sandwich assembly was established by slicing full logs and measuring color immediately after slicing. Full logs with intact casing were removed from corrugated combo storage bins covered with lids (Figure 3.11) in dark storage at approximately 34°F, the casing removed, and product sliced on a Hobart slicer model # 3913 (Troy, Ohio) at room temperature (approximately 70°F).



**Figure 3.11** Storage container for smoked ham logs

The ham was sliced to approximately 0.30 oz. per slice (to replicate the weight and thickness of a slice on the sandwich), and stacked seven slices tall and placed on a white cutting board (7 slices = approximately 10 mm thick). The test was repeated three times to establish consistency over a period of time (3 years). The first test (in 2012) was selected from three locations on the log (Figure 3.12).



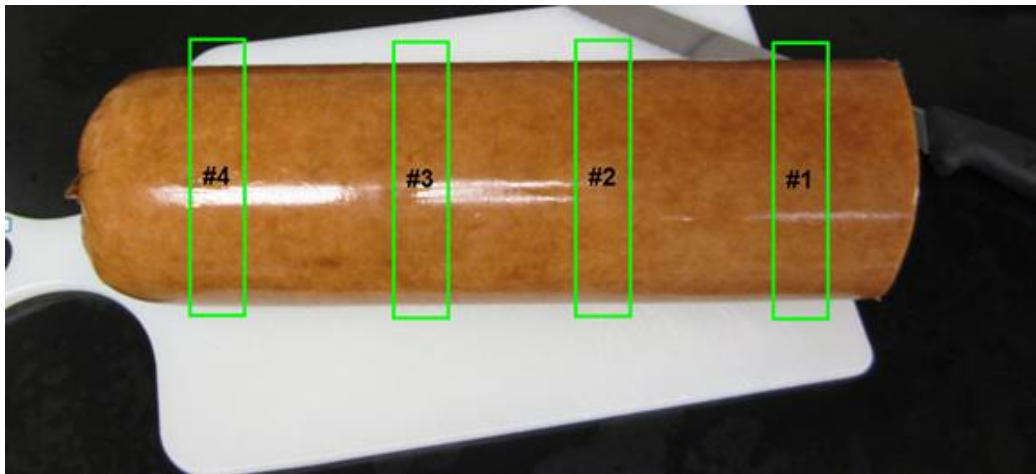
**Figure 3.12** Locations of fresh ham sampled for CIE  $L^*a^*b^*$  color measurements in 2012.

The first benchmark of initial  $a^*$  values ranged from 17.87 to 20.06 (dependent on location in the log). The results are found in Table 3.7.

**Table 3.7** Fresh sliced ham CIE  $L^*a^*b^*$  scores prior to assembly in 2012 (Product 12 days old at the time of slicing) Batch resulted in a min/max difference of  $a^* = 2.19$

<b>Date Collected</b>	5/24/2012		
<b>Ham Lot Code</b>	20120512 (produced on 5/12/2012)		
	product 12 days old		
<i>R&amp;D Lab</i>	#1 nearest the center of log	position #2	#3 near end of log
L* (1)	61.2	61.14	62.15
a* (1)	20.24	19.67	19.66
b* (1)	8.2	7.97	8.03
L* (2)	60.78	60.6	64.98
a* (2)	20.24	20.06	17.52
b* (2)	8.42	7.68	8.04
L* (3)	61.46	59.65	66.32
a* (3)	19.69	20.32	16.43
b* (3)	8.54	7.53	8.04
<b>L* AVERAGE</b>	61.15	60.46	64.48
<b>a* AVERAGE</b>	20.06	20.02	17.87
<b>b* AVERAGE</b>	8.39	7.73	8.04

The second attempt at benchmarking initial color was completed in 2014 and selected from four locations in the log (Figure 3.13).



**Figure 3.13** Locations of fresh ham sampled for CIE  $L^*a^*b^*$  color measurements in 2014 and 2015

The  $a^*$  values from the second benchmark of starting color ranged from 14.25 – 19.98. (Table 3.8)



**Table 3.8** Fresh sliced ham CIE  $L^*a^*b^*$  scores prior to sandwich assembly in 2014  
(Product 23 days old at the time of slicing). Batch resulted in a min/max difference of  $a^*$   
= 5.73

<b>Date Collected</b>	11/19/14			
<b>Link to photos</b>	<a href="#">2014-11-19 Colorimeter measurement of fresh ham</a>			
<b>Ham Lot Code</b>	1027 (produced 10/27/14)			
<i>R&amp;D Lab</i>				
	<b>#1 nearest the center of log</b>	<b>position #2</b>	<b>position #3</b>	<b>#4 near end of log</b>
$L^*$ (1)	69.53	60.71	60.30	60.31
$a^*$ (1)	14.26	18.80	18.54	19.30
$b^*$ (1)	8.16	7.10	7.03	6.65
$L^*$ (2)	68.91	61.71	58.47	58.64
$a^*$ (2)	14.24	18.91	19.34	20.37
$b^*$ (2)	8.07	6.72	6.71	6.75
$L^*$ (3)	68.66	61.78	58.10	58.04
$a^*$ (3)	14.26	18.78	19.54	20.28
$b^*$ (3)	7.75	7.06	6.94	6.79
<b><math>L^*</math> AVERAGE</b>	69.03	61.40	58.96	59.00
<b><math>a^*</math> AVERAGE</b>	14.25	18.83	19.14	19.98
<b><math>b^*</math> AVERAGE</b>	7.99	6.96	6.89	6.73

The third benchmark occurred in 2015 and was selected from four locations in the log. (Figure 3.13) The  $a^*$  values from the third and final benchmark of initial color ranged from 12.92 – 14.10 (Table 3.9).

**Table 3.9** Fresh sliced ham CIE  $L^*a^*b^*$  scores prior to sandwich assembly in 2015 (Product 28 days old at the time of slicing). Batch resulted in a min/max difference of  $a^* = 1.18$

Date Collected	5/20/15			
Ham Lot Code	20150422 (produced 4/22/15)			
	product 28 days old			
<i>R&amp;D Lab</i>				
	#1 nearest the center of log	position #2	position #3	#4 near end of log
$L^*$ (1)	58.76	57.76	56.96	54.74
$a^*$ (1)	12.45	13.18	13.38	14.50
$b^*$ (1)	5.18	5.19	4.86	4.69
$L^*$ (2)	60.30	60.12	58.36	56.69
$a^*$ (2)	12.68	12.52	13.28	13.82
$b^*$ (2)	5.52	5.83	5.09	4.67
$L^*$ (3)	57.14	59.20	56.49	55.74
$a^*$ (3)	14.50	13.07	14.05	13.97
$b^*$ (3)	6.02	5.83	5.40	4.82
<b><math>L^*</math> AVERAGE</b>	58.73	59.03	57.27	55.72
<b><math>a^*</math> AVERAGE</b>	13.21	12.92	13.57	14.10
<b><math>b^*</math> AVERAGE</b>	5.57	5.62	5.12	4.73

The results of the three attempts to benchmark initial ham tristimulus color scores demonstrates a large range of variability that can be seen from batch to batch. Because the ham formulation is made of both inside and outside muscles which vary in color and contains white fat deposits, the starting ham color has been established to range as much as  $\Delta a^* = 5.73$  within the same log (Table 3.8), and over time as much as  $\Delta a^* = 7.14$  (With a range of  $a^*$  scores of 12.92 (2015) to 20.06 (2012)). The inside ham muscle (semimembranosus) use for the Deli Express® ham has a small muscle (gracilis) lying over the top (also is referred to as the cap in the industry). Gracilis typically has more pigment; resulting in the darker red appearance. The chromameter factors all of these color variations into the color calculation along with any discoloration from photo-oxidation of the meat pigment. Slices with the darker red spots will result in higher  $a^*$  calculations, slices with white fat deposits will result in lower  $a^*$  calculations.

### **3.12 Gas measurement**

A Mocon Pac Check ® 650 head space gas analyzer was used to record oxygen and carbon dioxide gas levels. (Mocon, Minneapolis, MN) The head space atmosphere of all sandwiches (from like positions in the cooler) was checked individually throughout the course of each 30 day study. A “sticky nickel” (foam piece with adhesive) is used to prevent any back flow of gases. Once a package was measured for gas content, the package was opened and the CIE  $L^*a^*b^*$  values were measured using the procedure described in section 3.10, the sample then discarded. The number of test conditions and specific days of shelf life used for each test condition are reviewed in chapter 4. For tests 6 – 11, oxygen headspace readings were taken weekly for each test condition. For each test condition on a specific day, lane A (nearest the light), Lane B and Lane C samples were removed from a single shelf and analyzed. If two tests conditions were being evaluated, that translated to 6 packages checked for oxygen levels on a single shelf life day.

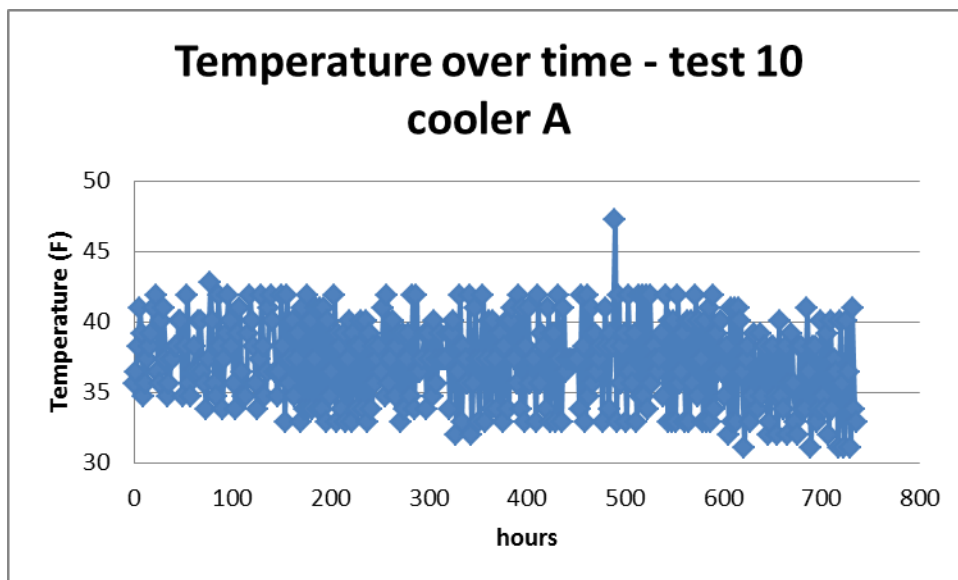
### **3.13 Case temperature monitoring**

Cooler temperatures were monitored using a DS1921G Thermochron iButton Device (Maxim integrated™, Sunnyvale, CA). Temperatures were recorded every two hours. Eleven tests were conducted, the number of coolers per test varied (Table 3.10).

**Table 3.10** Summary of number of coolers used during the 11 tests

Test number	number of coolers utilized in the test.	i-button temperature test performed.
1	5	yes
2	3	yes
3	3	yes
4	2	no
5	3	no
6	1	no
7	3	no
8	2	no
9	4	no
10	6	yes
11	2	yes

The coolers used in tests 2 and 3 were the same units used in tests 4-9. Cooler settings were not changed in tests 2-9. Cooler temperature tracking was established in test 2 and 3 and not tracked in tests 4-9. In test 10, additional coolers were added, so temperature tracking was used to verify the temperatures of the added coolers. Raw data for all of the coolers tracked are in Appendix A-C, J, K. Figure 3.14 shows an example plot containing all the data for one cooler during Test 10.



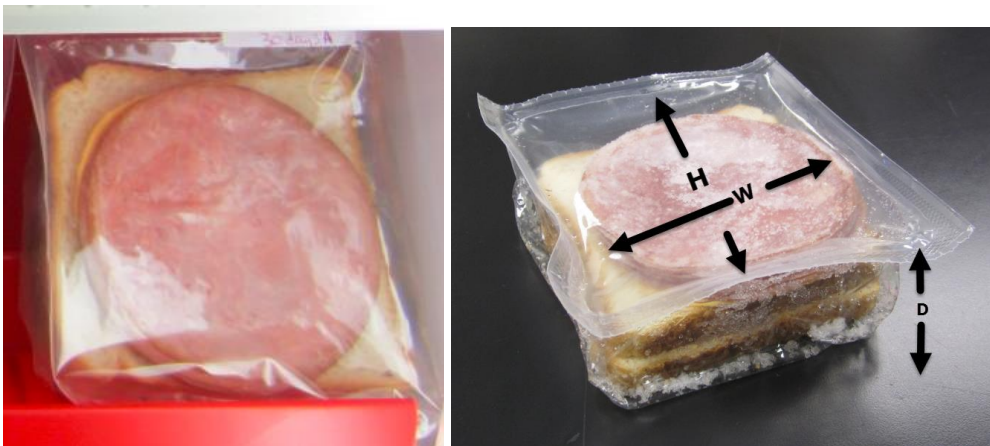
**Figure 3.14** Temperature over time for cooler A used in Test 10

### 3.14 Ham slicing and sandwich assembly procedure

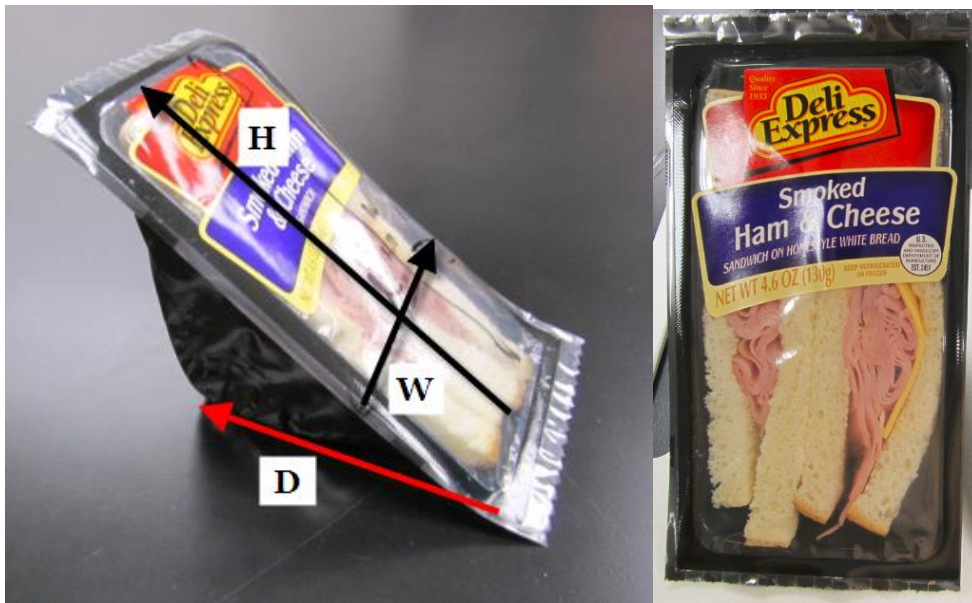
For preparation of the flat faced ham samples (Figure 3.15), ham was removed from refrigerated storage (30-34°F) and sliced at room temperature with a Hobart slicer (Troy, OH) to approximately 1/16" thick with an individual slice weight of 0.24 - .30 oz.

Within 30 minutes of slicing, two slices of bread, followed by one slice of cheese and a stack of seven slices of ham with an approximate total weight of 2.1 ounce (overlapping as closely as possible) were placed in the MAP packaging, gas flushed, sealed and stored frozen in a corrugated box with no light exposure. The maximum exposure to light prior to dark frozen storage is 1 hour. The average frozen dark storage was two weeks. The shape of the flat faced sandwich was square with an approximate dimension of 4" (H) x 4" (W) x 1.5" (D) = 24 in<sup>3</sup>. The packaging is approximately 4.5" (H) x 4.5" (W) x 2" (D) = 42.75 in<sup>3</sup>. (Figure 3.14) The product to package ratio is 1: 1.8. (24 in<sup>3</sup> product / 42.75 in<sup>3</sup> package)

The early tests (1-5) and the consumer study used samples that utilized "bunched" ham (figure 3.16). Bunching was achieved by shingling 7 – 9 slices of ham and placing "fluffed" on the bread. Bunched ham samples were assembled on a production line at E.A. Sween Company and water sliced using a high pressure water knife. The shape of the bunched ham samples are wedge shaped with an approximate dimension of 5.5" (H) x 3" (W) x 3.75" (D). The shape of the package is 5.5" (H) x 3" (W) x 3.75" (D). (Figure 3.15) The product to package volume ratio is 1:1. (61 in<sup>3</sup> product / 61 in<sup>3</sup> package)



**Figure 3.15** Example of a flat facing ham sample



**Figure 3.16** Example of a “bunched” ham sample in the wedge shape format

### 3.15 Gases used and starting oxygen levels

The target gas ratio is 79.5% nitrogen (Product specification in Figure 3.18), 20% carbon dioxide (Product specification in Figure 3.17), and  $>0.5\%$  oxygen after sandwich assembly and prior to freezing.

Test Parameter	Specification	Result
Purity (Zahm Nagel)	Min. 99.9%	99.99%
Total Sulfur (TS Analyzer)	Max. 100 ppb	25
Total Hydrocarbons	Max. 20 ppm	0.0
Dew Point	< -67 Degrees F.	-120.0
Moisture Content	Max. 19.20 ppm	0.25
Benzene	Max. 20 ppb	0
Acetaldehyde	Max. 200 ppb	0
Taste	none present	none present
Appearance	Normal/Colorless	Normal/Colorless
Odor	Normal/Characteristic	Normal/Characteristic

**Figure 3.17** Carbon dioxide specification (T.Bunde, personal communication, November 3, 2014)

Component	Specification
Nitrogen (N <sub>2</sub> )	>99.998%
Moisture (H <sub>2</sub> O)	<5 ppm
Oxygen (O <sub>2</sub> )	<8 ppm

**Figure 3.18** Nitrogen specification (T.Bunde, personal communication, November 3, 2014)

The typical O<sub>2</sub> starting point is parts per million (ppm) to 0.5 %. Because of the vacuum method used on the Multivac (section 3.19), the variability of starting O<sub>2</sub> in the package immediately following package sealing is as much as 0.183% (Table 3.11)

**Table 3.11** Initial O<sub>2</sub> levels (n=20) in the headspace immediately following manufacturing (samples from 5/4/15 production run at 9 am)

CO2 %	O2 %	CO2 %	O2 %
21.00	0.112000	20.30	0.000780
21.00	0.009430	20.70	0
20.70	0.183000	20.30	0
20.50	0.002820	19.70	0
21.00	0	20.20	0
20.50	0	19.80	0
20.30	0.001130	20.70	0.000090
20.40	0	20.10	0.005380
20.40	0	20.30	0.001260
20.20	0.000040	20.20	0

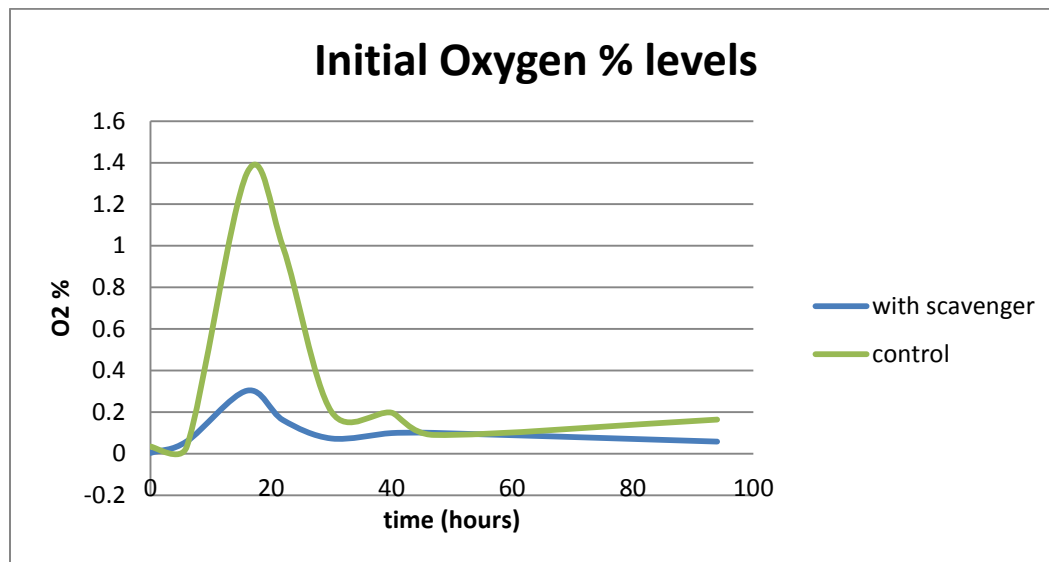
Average 0.015797  
 min 0.000000  
 max 0.183000  
 diff  
 (max -  
 min) 0.183000

Oxygen values of the samples post assembly for the first 94 hours in the freezer reveals a release followed by an absorption of trapped oxygen. Post packaging oxygen values without an oxygen scavenger achieved a maximum 1.35% residual O<sub>2</sub> at 16 hours (Table 3.12). Samples with an oxygen scavenger present reached a maximum 0.303% residual O<sub>2</sub> (Table 3.12, Figure 3.19).



**Table 3.12** Oxygen levels during the first 4 days in frozen storage (taken from 7/28/14 production). This product was used in the 10/26/14 consumer test

Oxygen levels during the first 4 days (in frozen storage)					
Sandwich production date 7/28/14					
Scavenger sachet			Control (no scavenger)		
time (hours)	CO2 %	O2%	time (hours)	CO2 %	O2%
0	23.5	0.0034	0	23.6	0.034
6	21.5	0.059	6	22.5	0.033
16	22.3	0.303	16	22.2	1.35
22	21.5	0.161	22	23.5	0.992
30	23.6	0.073	30	24.2	0.202
40	23.9	0.099	40	25.1	0.197
47	24.3	0.1	47	26	0.09
94	25.1	0.058	94	25.6	0.164
Average hours 30 - 94		0.0825 % O2	Average hours 30 - 94		0.16325 % O2



**Figure 3.19** Initial oxygen levels post assembly

A follow up review of oxygen levels after 17.5 hours frozen storage on a separate production run (current package only) also revealed a package with 1.66% oxygen in the headspace followed by a decrease in other sandwiches from the production run to less than 0.5% after 21.5 hours (Table 3.13).



**Table 3.14** Strength of light reaching the product (in lux). To convert lux to watt, multiply lux value by 0.0079. 1550 lux was the highest reading on a sandwich (12.2 watt of a 32 watt bulb)

Position	Lane C (lux)	Lane B (lux)	Lane A (lux)	(4050 lux) light source
	approx. 6.5" from light source	approx. 3.5" from light source	approx. 2" from light source	
Flat	207	290	450	
Angled	392	763	1550	

### 3.17 Coolers and product placement

Six Beverage-Air (Winston-Salem, NC) hinged glass door Lumavue™

MERCHANDISER SERIES LV27 coolers were used in this study. The coolers and bulbs varied in age. Each unit was set up to have 8 shelves, each containing the standard Deli Express plastic display tray underneath the product. The coolers were illuminated 24 hours/day. The light source was located on the right hand side of the cooler only. The product nearest the light source had an approximate distance of 2" from the light source. (Figure 3.20) The three lanes nearest the light source were utilized for testing in tests 6-11. (Figure 3.20) Placement in the cooler for tests 1-5 are described in Chapter 4.



**Figure 3.20** Cooler and product placement appearance in Tests 6-11

### **3.18 Light source**

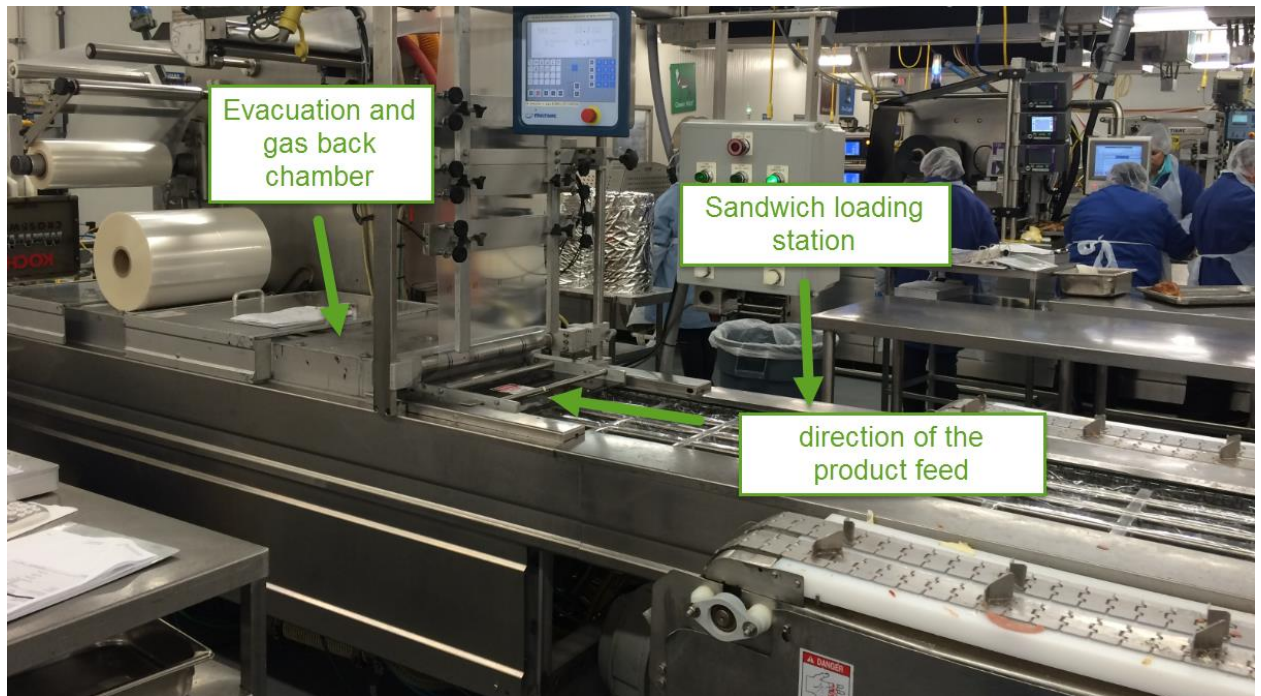
Unless otherwise noted, the bulb used was a Buyers Choice cool white 32 watt fluorescent bulb (Figure 3.21). This is the standard replacement bulb for Deli Express<sup>®</sup>. The age of the bulbs varied in Tests 6-9. For Test 10, new bulbs were installed in all coolers.

Technology: Fluorescent	Kelvin: 4100k
Kelvin Description: Cool White	Average Life (hours): 20000
Average Life (Years): 18.26	Dimmable: no
Lumens: 2550	Wattage: 32
Volts: 120v	Lumens Per Watt: 79.68
CRI: 75	Outdoor Rated: no
Returnable: No	Bulb Diameter (In.): 1
Bulb Type: T8	Base Type: Medium Bipin
Commercial/Residential: Commercial or Residential	Common Applications: Fluorescent fixtures
Package Quantity: 12	Energy Star Compliant: No
Estimated Yearly Energy Cost: 3.85	

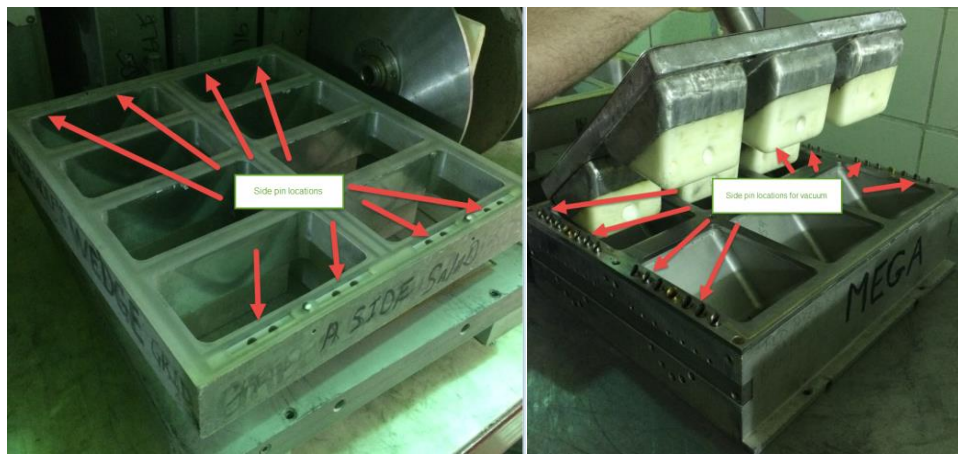
**Figure 3.21** Product specifications for fluorescent bulb used in coolers supplied to the retailer

### 3.19 Multivac packaging machine

Multivac R530 model is a horizontal form-fill-seal thermoforming machine. A roll of forming film is passes over a forming tool and with heat and plug assist, a pouch is formed. Product is loaded into the formed pouches and advances at a speed of 100 per minute (Figure 3.22). The upper non-forming film is applied to the filled pack cavities in the sealing die. Prior to sealing, 98% vacuum is applied to the chamber followed by gas injection into chamber / food pouch using a 79.5% N<sub>2</sub> / 20% CO<sub>2</sub> /.5 O<sub>2</sub> gas blend. The vacuum process is accomplished by piercing the lower film and pulling a vacuum through “side pins” (hollow ports). There are 6 side pins per row. In the case of the single wedge, there are two rows, 4 units per row. There is some variation in the amount of vacuum pulled across each individual compartment as the end compartments are closer to the side pins (Figure 3.23).



**Figure 3.22** Multivac machine used to package Ham & Cheese sandwiches



**Figure 3.23** A) Example of die tool and side pins for pulling vacuum on a Multivac (Mega size – 2 rows, 3 across). B) Example of the single wedge die (2 rows – 4 across)

After, the films are sealed hermetically to each other by means of a seal seam, followed by a cross cutting of the packages into individual units. Both square shape and wedge shape packaging was utilized.

### 3.20 Statistical analysis for CIE $L^*a^*b^*$ scores

All  $L^*$  and  $a^*$  values were compared for statistical differences using the Reaction Kinetics 8334 spreadsheets developed by Dr. Ted Labuza (University of MN). Using the Labuza method of kinetic modeling, allows loss of a food quality attribute to be analyzed (Equation 3.1).

Equation 3.1 (Labuza, 1984)

$$\frac{-d[A]}{dt} = k_f'[A]^n$$

Where A is the quality factor (color)

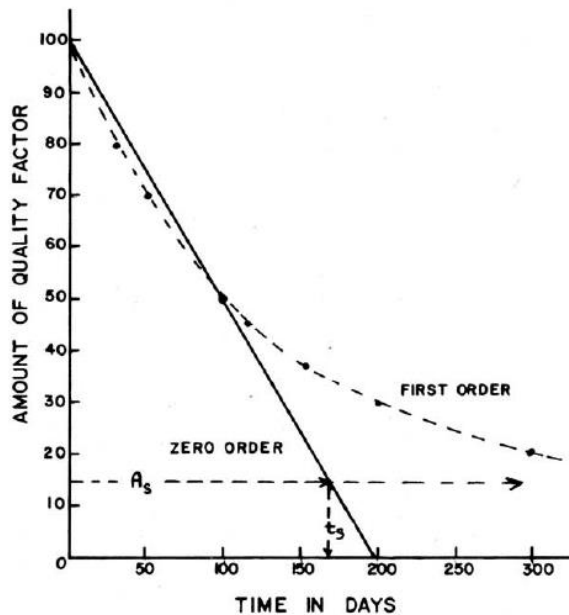
k is the rate constant.

t is time.

n is the reaction order.

From a data manipulation standpoint, most literature data for change in food quality (based either on some chemical reaction, microbial growth, death, or sensory value) follow a zero-order ( $n = 0$ ) or first-order ( $n = 1$ ) reaction model. (Labuza, 1984)

When the quality measure is plotted on the y-axis versus time on the x-axis, a zero order reaction can be used to draw a best fit line that is linear. (Figure 3.24) A first order reaction does not follow a linear pattern unless plotted on semi-log graph paper. (Labuza, 1984)



**Figure 3.24** Loss of a quality attribute as a function of storage time (Labuza, 1984)

Labuza demonstrated in 1984 that this method allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides. In addition, the visual average value is within the limits of both statistical methods. (Labuza, 1984)

The spreadsheets include statistical methods of  $R^2$  values and 95% confidence limits. Statistical differences are established by demonstrating non-overlapping areas at the 95% confidence limits between compared conditions.

### 3.21 Statistical methods for the consumer test

For the consumer test conducted in test 10, JAR (Just About Right), liking and intensity, meets expectation, purchase intent, and preference were used. The responses to the closed ended questions (Appendix J.14) were converted to a numerical interpretation, averaged, and analyzed using Analysis of variance (ANOVA) with means separation (Fisher's LSD) to characterize differences among the age pair samples (SAS version 9.4, SAS Institute, Cary, NC)



### 3.21.1 Liking questions

Liking questions were based upon a 9-point scale, where 1 = dislike extremely and 9 = like extremely. Intensity questions were based upon a 5- or 7-point scale, with scale anchors question specific. For liking and intensity questions, results are presented as mean scores.

For a specific question (row), values not sharing an uppercase letter are significantly different at the 95% confidence level ( $p < 0.05$ ). For a specific question (row), values not sharing a lowercase letter are significantly different at the 90% confidence level ( $p < 0.1$ ). Rows without letters indicate no significant difference.

### 3.21.2 Meets Expectations, Purchase Intent, and Preference Questions

For Meets Expectations questions, analysis was run for top 2 box and bottom 2 box scores. Top 2 and Bottom 2 box scores is a prevailing method used to reporting consumer attribute satisfaction scores (Example in Figure 3.25). The Top 2 box score represents the percentage of consumers who selected “Somewhat Better Than Expected” and “Much Better Than Expected” as a response to a question on a specific attribute. The Bottom 2 box scores represent the percentage of consumers who selected “Much Worse Than Expected” and “Somewhat Worse Than Expected” as a response. This allows the researcher to understand the percentage of participants who were satisfied on the attribute, and dissatisfied on the attribute.

For Purchase Intent, analysis was run for top 2 box scores. For Meets Expectations, Purchase Intent, and Preference, reported values are percentages of consumers. Values are subject to rounding error. Values not sharing an uppercase letter are significantly different at the 95% confidence level ( $p < 0.05$ ). Values not sharing a lowercase letter are significantly different at the 90% confidence level ( $p < 0.1$ ). Values without letters indicate no significant difference.

xx. Overall, how well does this product meet your EXPECTATIONS of a pre-packaged sandwich?



**Figure 3.25** Example of a question using the Top 2 and Bottom 2 box score method

This is a prevailing method used to reporting attribute satisfaction scores. The advantage of this method is it includes the Top 2 box scores represents the percentage of consumers who selected “Somewhat Better than Expected” and “Much Better than Expected” as a response. The Bottom 2 box scores represent the percentage of consumers who selected “Much Worse than Expected” and “Somewhat Worse than Expected” as a response.

### **3.21.3 JAR (Just About Right) Questions**

JAR Scores are based on a five-point JAR scale collapsed into three categories, where 1 = not enough, 3 = JAR, and 5 = too much of an attribute, with scale anchors question specific. Reported values are percentages of consumers. Values are subject to rounding error. For JARs 70% or greater (and less than 20% TL (“too little”) or TM (“too much”), the attribute can be considered sufficiently optimized.

### **3.21.4 Penalty analysis in consumer testing**

Penalty analysis is represented only for attributes that were rated as “Too Little” (TL) or “Too Much” (TM) by at least 20% of the respondents. A penalty score  $\geq 0.50$  is considered top penalty;  $\geq 0.25$  and  $< 0.50$ , middle penalty; and  $< 0.25$ , bottom penalty.

Penalty analysis is used by researchers to gain insight on JAR (Just About Right) responses of the product attributes that most affect liking, purchase interest or any other product-related measure. In this study, the question “Rate the meat color of this product” was the main diagnostic question. The choice was made to phrase the responses to this question as “too light” or “too dark” as the anchor responses. This choice was made based on the belief that this concept / verbiage were more easily understood by the consumer. The alternative was to use terms like pink/red as acceptable vs. brown/grey which positioned the question as being more negative. The open ended question “What was the main reason you preferred this sample” was included to allow consumers to

express comments or descriptions on meat color. Product attributes used in penalty analysis are measured with “Just -about-right” (JAR) scales. JAR scales collapse a 5 point scale to 3 point scales and helps give the researcher direction on areas of concern when 20% of the respondents rate an attribute on either side of the JAR scale (too much or not enough). When JARs results are high (~70%) of the responses as “Just About right”, this is an indication of the attribute being sufficiently optimized. Based on years of product testing, 70% can be used to indicate whether a product is fully optimized. This guideline is not correlated to in-market performance. JAR responses are used to help determine what attributes are affecting overall liking scores and key measures.

## **4 Preliminary evaluations: Tests 1 – 9**

Nine initial tests were conducted to better understand meat color performance of the bestselling Deli Express<sup>®</sup> Ham & Cheese sandwich (Mintel, 2014), compared to potential improvements to product and packaging. We explored multiple potential solutions to prevent or slow meat discoloration of ham on a refrigerated sandwich within a process of gas flush (MAP), frozen storage, followed by 30 day refrigerated shelf life. The potential solution(s) needs to be practical and cost effective for E.A. Sween Company (EAS) to implement. Keeping the product visible to the consumer is a must to meet the consumer expectation upon seeing the product. Because the retail refrigerated storage equipment is not consistently in the control of EAS, the food and packaging are areas of focus, with packaging as a primary focus due to the sales success of the current Ham & Cheese sandwich formulation. Understanding how the product performs under LED lights is also of interest as it is frequently asked if this solution can help.

EAS knows discoloration occurs based on retailer operator input and EAS employee observations in store, but has not collected formal data around frequency of occurrence. Very little discoloration is reported by consumers to the company. This could be in part due to the practice of both EAS and retail store employees in removing the product from the shelf as needed. A belief at EAS is that the discoloration is a result of to being too close to the light source in the refrigerator, and that open coolers, because of temperature abuse, cause greater discoloration. Standard practice today is to instruct EAS and retail staff to place sliced meat sandwiches away from the light source when possible. This strategy is limited as often the choice for retail shelf space is limited, and there is not conclusive proof that all discoloration occurs nearest the light source.

The results and conclusions of the exploration are reviewed in the following sections 4.1 – 4.9. The learnings from these evaluations were used to create a final study designed for consumer testing. The consumer input test is evaluated in chapter 5.

## **4.1 Impact of light source and cooler**

### **4.1.1 Overview of Test 1**

The purpose of this trial was to establish the impact of cooler type (open air versus several brands of closed door) and the light source (LED versus Fluorescent) on ham color. Deli Express<sup>®</sup> sandwiches are stored in a variety of refrigerator brands and styles. In some store accounts, the refrigerator is provided by E.A. Sween Co. (EAS) (Cooler D in Table 4.1 is the model currently being provided to the industry), but often product is placed into available coolers and space that is already at the retail location. The idea to investigate an open cooler versus a closed door cooler is based on the store operator and EAS sales staff beliefs that open coolers run warmer, and that the warmer the temperature, the more likely discoloration is to develop. The American Meat Science Association (AMSA) supports the premise that display temperature can affect meat color stability (Hunt et al., 2012), however the Nannerup et al. study for cured, cooked ham in Modified Atmosphere Packaging (MAP) took into account the interaction of five critical parameters 1) percent residual oxygen (O<sub>2</sub>) in the package, 2) product to headspace volume ratio in the package, 3) temperature of the cooler, 4) light intensity, and 5) packaging O<sub>2</sub> transmission rate (OTR), they concluded that color stability of ham was not affected significantly by a temperature change (5°C to 10°C) by measuring color with a Minolta Chroma meter through the packaging at twelve days throughout a thirty-four day study (Nannerup et al., 2004).

The light source in the cooler was investigated because store operators are exposed to information from light bulb manufacturers that convey the idea that eliminating ultraviolet light (UV) will reduce discoloration. Low UV light bulb manufacturers promote awareness that discoloration of deli lunch meats can be solved by the light source used (Promollux.com). Other light bulb manufacturers report that the available literature on the impact of the light source is inconsistent (Sylvania, 2014). For fresh raw meats, other researchers have found that color stability was improved with use of a UV filter (Fresh sausages in MAP, measured with a spectrophotometer (Martinez et al., 2006)) as well as display under LED lights (fresh beef in MAP, measured with a

spectrophotometer (Steele, 2009)). For cured ham in MAP, photo-oxidation of the meat pigment has been demonstrated to depend linearly on the O<sub>2</sub> content in the package for both visible (436 nm) and UV-light (366 nm) (Møller, Bertelsen, and Skibsted, 2002). This would suggest that a light source with no or limited UV output would not reduce ham discoloration if visible light is still present.

While the variables of the cooler type and light bulb used are not always in control of EAS, an understanding of the impact is important as it is frequently mentioned as a potential solution by stores, and the stores look to EAS to give them best storage practices to follow. A variety of coolers and light sources were evaluated (Table 4.1). The coolers were selected based on equipment owned by EAS and are models that are readily available to the convenience store industry. EAS provides approximately 300 new coolers to retail partners per year. However many EAS customers are using different cooler space to store Deli Express<sup>®</sup> sandwiches.

**Table 4.1** Cooler and light source for refrigerators evaluated in Test 1

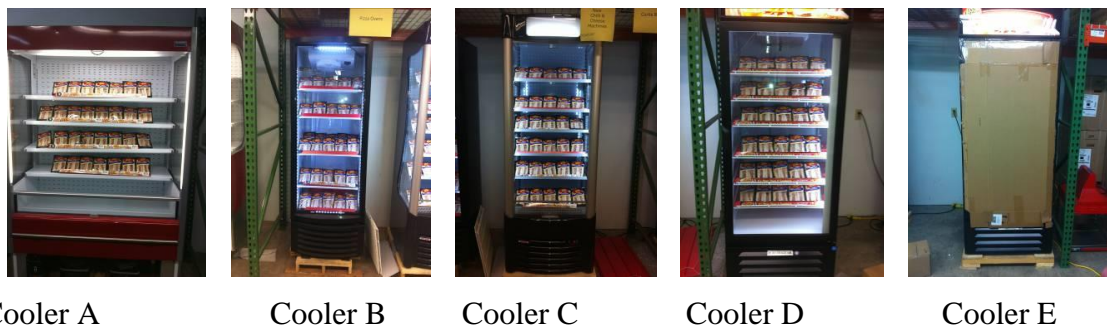
Cooler designation	Cooler type	location	open or closed cooler	Bulb type	Bulb location
A	Ojeda, model # ALPA-120-G2	Spartanburg, SC	open air	25 watt fluorescent bulb, Philips brand	overhead only.
B	Criotec model #CFX-11 BM LV LE	Santa Catarina; Nuevo Leon, Mexico	closed door	LED-tech, 9V	top, bottom, both sides
C	AHT, model AC-M	Rottenman, Austria	open air	LED panel, 52 watt OT-12	top, bottom, both sides
D	Beverage-Air hinged glass door Lumavue™ LV27 c	Winston-Salem, NC	closed door	Buyers Choice cool white 32 Watt fluorescent bulbs	right side only
E	Beverage-Air hinged glass door Lumavue™ LV27 c	Winston-Salem, NC	closed door	no bulb	-

#### 4.1.2 Methods and Materials Test 1

The cooler set method was to fill all shelves; one sandwich deep, in each of the five coolers listed in Table 4.1 (above) and evaluate one sandwich from each cooler from the same position on the shelf throughout thirty one days of refrigerated storage. A total of thirty sandwiches were placed per cooler, with twenty-one sandwiches used in the evaluation (See Table 4.2 for sample location in each cooler and Figure 4.1 for the visual appearance of the coolers at the start of the study).

**Table 4.2** Test 1 cooler set up. A) Diagram of cooler set (grey shaded samples not evaluated) B) Sample numbers and corresponding day in shelf life

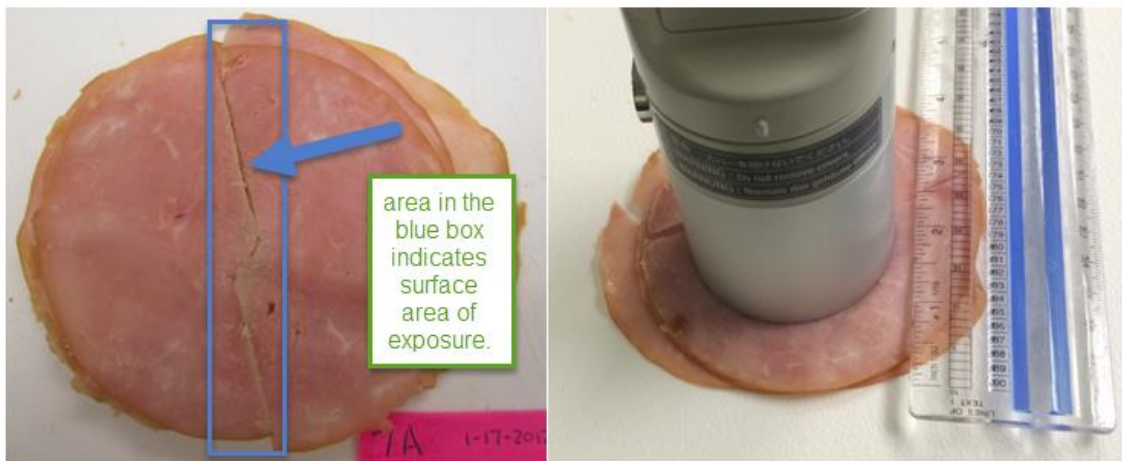
Cooler Sample Configuration			Day	Date	Sample
Cooler A	A	Cooler A Current model open air cooler	Day 3	1/9/2012	1
		light	Day 4	1/10/2012	2
		light	Day 5	1/11/2012	3
		light	Day 6	1/12/2012	4
		light	Day 7	1/13/2012	5
Cooler B	LED	Cooler B - LED bulbs	Day 10	1/16/2012	6
		LED	Day 11	1/17/2012	7
		LED	Day 12	1/18/2012	8
		LED	Day 13	1/19/2012	9
		LED	Day 14	1/20/2012	10
Cooler C	LED	Cooler C LED	Day 17	1/23/2012	11
		LED	Day 18	1/24/2012	12
		LED	Day 19	1/25/2012	13
		LED	Day 20	1/26/2012	14
		LED	Day 21	1/27/2012	15
Cooler D	Current	Cooler D Fluorescent	Day 24	1/30/2012	16
		Fluorescent	Day 25	1/31/2012	17
		Fluorescent	Day 26	2/1/2012	19
		Fluorescent	Day 27	2/2/2012	20
		Fluorescent	Day 28	2/3/2012	21
Cooler E	Current - No Light	Cooler E Fluorescent model - no light	Day 31	2/6/2012	22
		Fluorescent model - no light			
		Fluorescent model - no light			
		Fluorescent model - no light			
		Fluorescent model - no light			



**Figure 4.1** Visual of coolers utilized in Test 1

On each color measurement day, “like” positions in the cooler were pulled for evaluation (for example on day 3, all number “1” samples were removed from the top shelf, far left (see Table 4.2B above for all day / sample number pairings)). All samples were checked for O<sub>2</sub> % in the package headspace using a Mocon analyzer (as described in section 3.12),

and measured for color using a Minolta Chroma Meter (as described in section 3.10). Sandwiches in this study were measured for  $L^*a^*b^*$  values using method 1 (Reconstructing and flattening “bunched” ham into a flat surface and measuring the center where the two half slices meet - Figure 3.9 in methods and materials). The  $b^*$  values are recorded in Appendix A.12 but are not evaluated for statistical differences as lightness ( $L^*$ ) and redness ( $a^*$ ) are the most important values for ham color measurements (Sheridan et al., 2007). In study one, only a single chromameter measurement was taken from the center of the ham surface. The diameter of the ham is approximately 4”. The diameter of the Chroma meter lens is 1.9685”. Using the area of a circle ( $a = \pi * R^2$ ), the lens captures approximately 24% of the ham slice surface area. Of the area measured, only 8% of the surface area contains surface exposed to light and  $O_2$  (approximately 1/8” wide by 1.9685” Figure 4.2 the area within the blue box). This measurement method is not ideal given the limited amount of exposed ham surface area to light and  $O_2$  being captured in the color measurement, however it is important to evaluate the retail appearance of the product as the consumer sees it, and use available equipment at EAS.

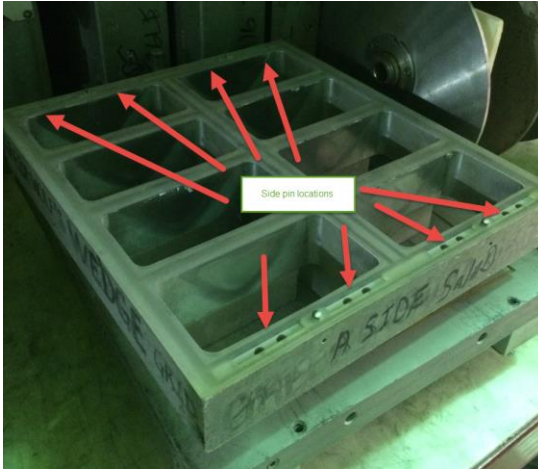


**Figure 4.2** Chromameter measurement area on ham surface

Ham color (once removed from the package) was also visually inspected at each color measurement day after day 5 (documented in Appendix A.1-A.10). Photos were taken to document visual color (Method in section 3.10).



The critical level of residual O<sub>2</sub> in MAP with sliced cured ham has been established to be between 0.1 and 0.5% (Møller et al., 2000). Because obtaining O<sub>2</sub> measurements from the head space of the package breaks the MAP seal (accomplished by piercing with a needle to obtain the gas sample for analysis), all samples were removed from the package for color measurement and visual inspection and then discarded. The growth of MAP with gaseous headspace between the meat and film has increased the difficulty of obtaining color measurements during display (Hunt et al., 2012). The advantages of removing the product from the package is that it allows the O<sub>2</sub> level of each package to be established (and the O<sub>2</sub> level is a critical measure) and it avoids the need to invert the sample to get the meat surface in direct contact with the package (which often causes moisture build up and smear on the package surface) (Hunt et al., 2012). The disadvantage of removing the meat from the package is that it does not allow repeated measures of the same location throughout the study, which introduces the potential of sample variation. AMSA recommends preparing multiple sub-samples out of the original sample batch as a method to use when establishing the O<sub>2</sub> level of each package is critical (Hunt et al., 2012). All sandwiches were produced and pulled from the same sandwich production lot to minimize differences in the materials used. (See section 3.1 for individual materials) The sandwiches used were all packaged within a five minute period of time. With one hundred and fifty sandwiches made and eight sandwiches gas flushed and sealed during each cycle of the machine, a total of 18.75 machine cycles were required to complete the packaging of the sandwiches. Figure 4.3 illustrates the design of the packaging forming station (referred to as a die). Each cycle of the machine creates 8 packages (1 cycle is the forming of the package, evacuation of the gas, followed by a replenishment of the desired gas blend). The vacuum ports are located on each side of the packaging tool.



**Figure 4.3** Example of the single wedge Multivac tool. Red arrows indicate location of the vacuum ports

#### 4.1.3 O<sub>2</sub> percentage per package Test 1

For each individual sandwich produced, there are three distinct timeframes / phases for the O<sub>2</sub> levels in the head space of the package. Phase 1 is the immediate level following the MAP process. Phase 2 is the level during freeze down and frozen storage. This phase can last up to 9 months for EAS. During this phase, trapped O<sub>2</sub> in the food diffuses into the head space of the package. The final phase 3 is the O<sub>2</sub> level during the 30 day refrigerated shelf life. The amount of O<sub>2</sub> in the headspace of each package during each of these phases has variation. In phase 1, the variation is primarily due to differences in the amount of vacuum applied for each package in the MAP process (As much as 0.183% per package - Section 3.15). Because each sandwich cycle of the Multivac<sup>®</sup> machine contains eight packages (2 rows of four) with the vacuum ports only on each side of the tooling (Figure 4.3), the middle four packages do not have an equal amount of vacuum pulled as the force of the vacuum is greater on the outer cavities and the inner cavities see a diminished vacuum pull.

In phase 2, the O<sub>2</sub> level variation increases (building off of the initial phase 1 variation) based on the amount of trapped air in the sandwich components. Because each bread slice varies in size and weight (from 0.9 to 1.3 oz. per slice), bread is believed to be a significant contributor of trapped air differences in this phase. Bread is very porous and

the pores are filled with air. Typically white bread has a porosity of 64.4 – 84 % with 99% of the pores connected (Wang, 2014). When the vacuum packaging process (approximately 3 seconds) lowers the O<sub>2</sub> level in the air space in the package, this creates a driving force that causes the O<sub>2</sub> level to increase in the headspace post-sealing as the gas flows out of the bread pores. O<sub>2</sub> levels in the headspace during phase 2 have been established to increase as much as 1.6% (Section 3.15). In phase 2, the O<sub>2</sub> levels increase post packaging and then fall within 24 hours (Figure 3.18, section 3.15). The inability to continually check the same package for O<sub>2</sub> over time makes this impossible to declare conclusively, but repeated checks during phase 2 have revealed at least one package has greater than 1.0% O<sub>2</sub> in the first twenty four hours (typically between 16-17 hours), and the checks beyond 24 hours have not revealed O<sub>2</sub> levels above 1.0%. The speculation is the higher O<sub>2</sub> packages represent one of the middle four cavities per cycle. Potential reasons for the changes in O<sub>2</sub> levels in phase 2 are discussed below.

In phase 3 (refrigerated storage), the oxygen levels typically start >0.5%, and proceed to zero over the course of the 30 days (O<sub>2</sub> consuming reactions discussed below). For study 1, the O<sub>2</sub> established in the headspace of the package at the start of phase 3 ranged from 0.12 to 0.57% (Table 4.3)

**Table 4.3** Day 1 measurements after 24 hours thaw in Test 1

Day 1 thaw samples	
Control O2 %	Control O2 %
0.35	0.190
0.12	0.244
0.43	0.298
0.359	0.470
0.572	0.366
	0.239
0.259	0.202
0.412	0.449
0.200	0.253
0.179	0.193
average	0.30
min	0.12
max	0.57

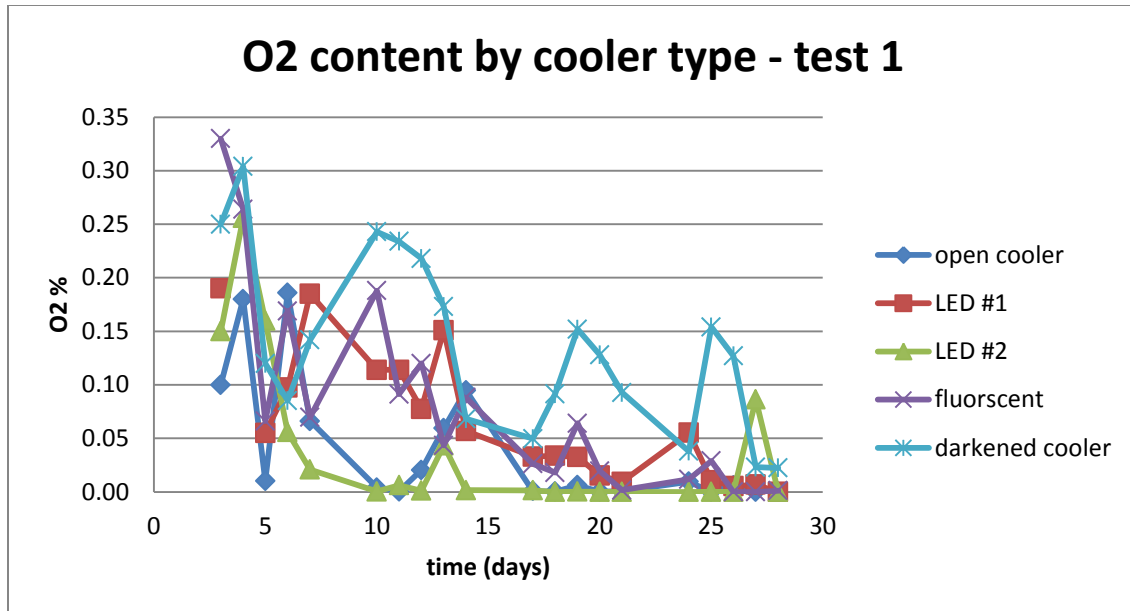
In phases 2 and 3, there are many potential reactions for O<sub>2</sub> to be consumed in. This includes 1) formation of multiple meat pigments (Hunt et al., 2012); 2) Interaction with antioxidants in the formula (Møller, Weber, and Bertelsen, 1999), 3) Lipid oxidation in

both ham (Anderson and Rasmussen, 1992) and cheese, 4) Aerobic microorganism consumption, and 5) pH changes (driving oxidation reactions). Given that formation of metmyoglobin is not the only source of O<sub>2</sub> consumption, this helps provide insight as to why O<sub>2</sub> levels appear to change over time even in a dark cooler, and why the differences in average O<sub>2</sub> between coolers may provide an indication of how much O<sub>2</sub> is consumed specifically by meat pigment changes. If other O<sub>2</sub> consuming variables are constant and light and meat pigment formation are the key differences, the differences in the average O<sub>2</sub> levels in the dark cooler compared to the others could directionally represent O<sub>2</sub> used in the formation of metmyoglobin.

The amount of O<sub>2</sub> available is critical because in fresh meat, the competition for O<sub>2</sub> between myoglobin and mitochondria determines the level of penetration beneath the meat surface and the thickness of the Oxy-myoglobin (OMb) layer (this is red in appearance) (Hunt et al., 2012). When partial pressures of O<sub>2</sub> are higher than atmospheric conditions in a package, a thicker OMb layer is formed. But under low O<sub>2</sub> partial pressures (<7 mm Hg), deoxygenation of OMb to DMb (Deoxy-myoglobin) is favored because dissolved O<sub>2</sub> in the muscle tissue is consumed, leaving DMb susceptible to oxidation by O<sub>2</sub> radicals and species (like Hydrogen Peroxide) (Hunt et al., 2012). DMb is more susceptible to conversion to Metmyoglobin (MMb) (Hunt et al., 2012). While the curing and cook process of ham results in the formation of the more stable color pigment nitrosylhemochrome, light is a catalyst in dissociation of Nitric Oxide (NO) from the cured meat pigment moiety, leaving it susceptible to conversion to metmyoglobin (Varnam and Sutherland, 1995). Light-induced oxidation of nitrosylhemochrome depends linearly on O<sub>2</sub> concentration, so the more O<sub>2</sub> present, the greater the potential for formation of metmyoglobin. (Møller et al., 2002) The variability of O<sub>2</sub> per package could help explain why apparently random sandwiches within a cooler set will show significant discoloration when others around it do not, as a result of the greater initial O<sub>2</sub>.

For EAS, the goal is to validate that each sandwich production lot is between 0 and 0.5% O<sub>2</sub> at the time of initial manufacture (phase 1). This is accomplished by random sampling and Mocon measurement of sandwiches throughout the run. Changes to the O<sub>2</sub> level during phase 2 (frozen storage) has not been tracked by EAS. Phase 3 O<sub>2</sub>

percentage is checked during refrigerated shelf life studies which has historically shown that very low O<sub>2</sub> is present (often measured in parts per million (ppm)). There is also the potential for “leakers” which are packages that do not have a complete seal or were damaged after packaging. Leakers are often caused by food debris in the seal area and are not always found in visual inspection. While the percent leakers is not tracked by EAS, occurrence is low with a maximum of 2% found during shelf life studies. Leakers are easily spotted in phase 3 as they result in significantly higher O<sub>2</sub> levels (greater than 1.0%, often approaching atmospheric O<sub>2</sub> levels (20.6%)) and visual discoloration. As noted before, the average O<sub>2</sub> percentage for the day one thawed sandwiches was 0.30% with a range of 0.12 – 0.57% (Table 4.3). In Test 1, the cooler without light (Cooler E) had the highest average O<sub>2</sub> percentage (0.14%) over the course of the thirty-one day refrigerated shelf life (Table 4.4 and Figure 4.4). This directionally could be a result of less O<sub>2</sub> being consumed to form metmyoglobin. The coolers A-D that were exposed to light averaged 0.04 – 0.08% residual O<sub>2</sub> percentages in the head space (Table 4.4) and had visually detected discolored ham (Appendix A.1-A.10) which could indicate O<sub>2</sub> was consumed in the presence of light to oxidize nitrosylhemochrome to metmyoglobin (Møller et al., 2000). The O<sub>2</sub> differences found in this study supports the importance of the combination of O<sub>2</sub> with both visible and ultraviolet light in the photo-oxidation mechanism (Anderson et al., 1988) as the dark cooler had more oxygen available, while the coolers exposed to light had less average O<sub>2</sub> levels.



**Figure 4.4** O<sub>2</sub> percentages per package over time (Test 1). Note that these are taken from different locations at each time (See Table 4.2)

**Table 4.4** O<sub>2</sub> content by cooler type (O<sub>2</sub> is listed as a percentage per package)

day	Cooler A	Cooler B	Cooler C	Cooler D	Cooler E
3	0.10	0.19	0.15	0.33	0.25
4	0.18		0.26	0.26	0.30
5	0.01	0.06	0.16	0.07	0.12
6	0.186	0.097	0.056	0.169	0.085
7	0.066	0.185	0.021	0.070	0.142
10	0.004	0.114	0.000	0.188	0.243
11	0.000	0.114	0.006	0.091	0.234
12	0.021	0.078	0.001	0.120	0.218
13	0.060	0.151	0.043	0.043	0.173
14	0.095	0.057	0.002	0.088	0.068
17	0.001	0.033	0.002	0.026	0.050
19	0.0002	0.034	0.000	0.018	0.0916
20	0.006	0.033	0.000	0.064	0.152
21	0.000	0.015	0.000	0.020	0.128
24	0.001	0.009	0.000	0.002	0.093
25	0.010	0.055	0.000	0.012	0.038
26	0.000	0.011	0.000	0.029	0.154
27	0.006	0.005	0.000	0.000	0.127
28	0.000	0.007	0.086	0.000	0.023
31	0.000	0.000	0.000	0.001	0.022
Average	0.04	0.07	0.04	0.08	0.14
minimum	0.00	0.00	0.00	0.00	0.02
maximum	0.19	0.19	0.26	0.33	0.30

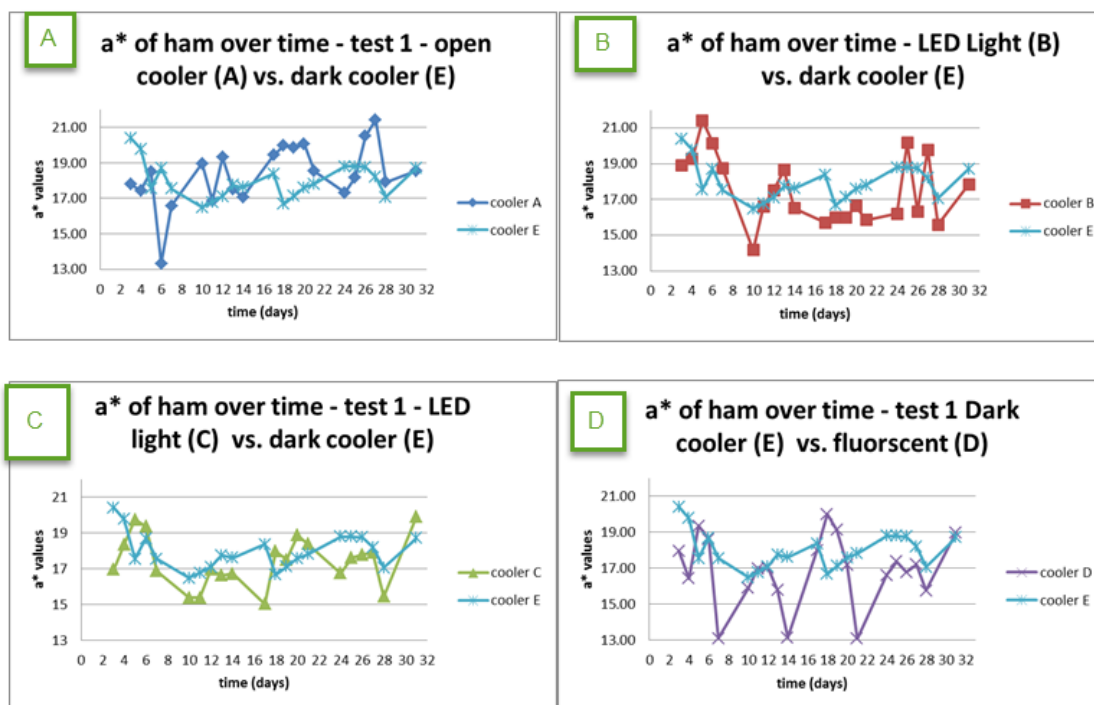
#### **4.1.4 $L^*a^*b^*$ analysis – Test 1**

A chromameter was used in conjunction with visual inspection to assign a numerical value to the color of the ham and have an objective measurement to complement the visual subjective evaluation. A chromameter uses CIE  $L^*a^*b^*$  co-ordinate system to assign XYZ values (3.10). A spectrophotometer is another device capable of measuring color, however from a practical standpoint, EAS has a chromameter as available equipment, and the portability of the device makes it easy for use in a manufacturing or retail setting. CIE tristimulus XYZ values are the most commonly used technique for evaluating cured ham color (Sheridan et al., 2007). The color scale for  $a^*$  value is  $a^* = 0$  represents true grey. The larger the positive  $a^*$  value is, the more red color that is present. A decreasing positive  $a^*$  value indicates a loss of redness (Konica Minolta). A negative  $a^*$  value represents green. The  $L^*$  scale is 1 to 100. A higher  $L^*$  value means greater fade (white), while a lower  $L^*$  value indicates darkening (Konica Minolta). The results of the chromameter test are shown in Table 4.5 and Figure 4.5.

**Table 4.5** Ham  $a^*$  value average, min and max over 31 days refrigeration Test 1

Sample						
number	day	cooler A	cooler B	cooler C	cooler D	cooler E
1	3	17.79	18.90	16.95	17.96	20.41
2	4	17.44	19.27	18.34	16.43	19.79
3	5	18.50	21.43	19.76	19.35	17.56
4	6	13.32	20.15	19.37	18.62	18.70
5	7	16.57	18.76	16.87	13.09	17.55
6	10	18.97	14.16	15.39	15.89	16.50
7	11	16.86	16.62	15.38	16.98	16.77
8	12	19.31	17.53	16.97	17.03	17.10
9	13	17.53	18.66	16.63	15.77	17.75
10	14	17.06	16.52	16.74	13.14	17.62
11	17	19.43	15.71	15.04	18.01	18.38
12	18	19.96	15.97	17.98	20.00	16.67
13	19	19.86	15.98	17.51	19.13	17.13
14	20	20.08	16.64	18.88	17.18	17.60
15	21	18.52	15.86	18.39	13.07	17.81
16	24	17.32	16.21	16.76	16.61	18.79
17	25	18.17	20.17	17.62	17.36	18.8
19	26	20.5	16.33	17.79	16.77	18.76
20	27	21.44	19.78	17.9	17.2	18.21
21	28	17.92	15.59	15.44	15.75	17.07
22	31	18.56	17.84	19.93	18.98	18.72
	average	18.34	17.53	17.41	16.87	17.99
	min	13.32	14.16	15.04	13.07	16.50
	max	21.44	21.43	19.93	20.00	20.41
	range	8.12	7.27	4.89	6.93	3.91





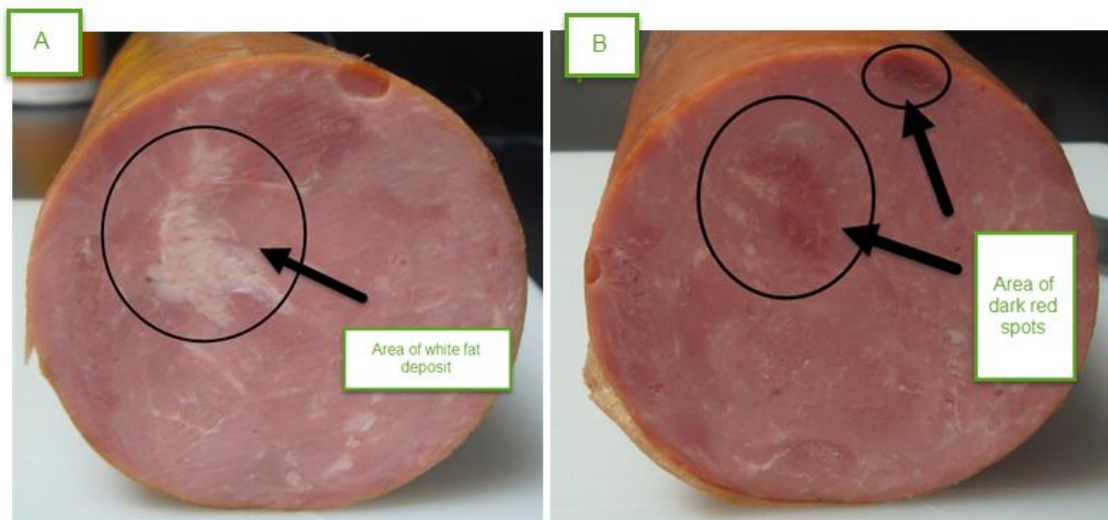
**Figure 4.5** comparison of  $a^*$  performance over time (days) A) Cooler A (open cooler) versus Cooler E (dark cooler). B) Cooler B versus Cooler E. C) Cooler C versus Cooler E. D) Cooler D versus Cooler E

A review of the results (Figure 4.5 A - D) of the  $a^*$  value over time for each cooler using all sandwiches reveals that the lowest  $a^*$  values occurred on samples that were closest to the light source (see Table 4.2 for cooler locations). This is an indication that the light intensity by close proximity to the product is important in metmyoglobin development. Over the 31 day evaluation period in this test, there was significant variability in  $a^*$  values over time within each cooler, including in cooler E (dark cooler) which should have been the most stable with no light exposure. In cooler E, ham sandwiches trended downward (decreasing) in  $a^*$  value days 3 to 10, followed by an increase on days 11 – 17, a decrease at day 18, followed by another increase through day 27, a decrease at day 28, and an increase at day 31 (Figure 4.5). Li et al. found in vacuum packed sliced ham a pattern where  $a^*$  values increased from day 1-7, decreased days 7-14, followed by an increase in  $a^*$  value through day 21. This study also used a Minolta Chroma meter to measure unwrapped ham (Li et al., 2012). This outcome of increasing and decreasing

values throughout the study is unlike dry salami where Yen et al. established a consistently decreasing  $a$  value over time (Yen et al., 1988). Differences could be attributed to the color measurement method. Yen et al. used a Gardner Color and color difference meter. The Gardner Color scale (ASTM D1544) is a single one dimensional number color scale ([www.lovibondcolour.com](http://www.lovibondcolour.com)). Li et al. also speculated that the Yen et al. outcome may have been due to a lower pH in the product as a result of lactic acid producing bacteria in the salami resulting in a faster breakdown of nitrite and enhanced oxidation (Li et al., 2012). In nitrite cured meats, nitrite is not the nitrosating species that reacts with myoglobin to form nitrosylhemochrome (Pegg and Shahidi, 1997) To get to the primary reactive species; nitrite must be reduced by ascorbate, which is a pH dependent reaction. The pH dependency is complex as nitrite / nitrous acid reactivity increases with decreasing pH, while ascorbic acid / ascorbate as a reductant increases as pH increases (Pegg and Shahidi, 1997). This pH dependent reaction may help explain volatility in  $a^*$  value in a MAP sandwich system. Each of the sandwich components has different starting pH levels (reported as 6.0 to 6.5 in ham, a maximum of 6.0 for cheese and 4.5 – 5.5 for bread (Table 3.1)). Over time, the pH of the individual components changes due to equilibration of the sandwich components with each other, and the production of lactate and hydrogen peroxide by lactic acid bacteria over time in the favorable MAP environment (Metaxopoulos, Mataragas, and Drosinos, 2002). In the case of smoked ham in this sandwich system, the pH of the meat has been demonstrated to decrease from 6.21 to 5.87 over time (Table 3.5) which could impact the reactivity of nitrite driving both reformation of nitrosylhemochrome, and breakdown of nitrosylhemochrome as the weak acid buffer systems shift throughout the shelf life. Within all individual coolers A-E in this study,  $a^*$  outcomes varied, without a consistent pattern emerging (Figure 4.5). In this study, cooler E is used as the control cooler to compare the others to. Cooler E had the smallest range in  $a^*$  values between the minimum and maximum ( $\Delta a^* = 3.91$ ) and also had the highest minimum  $a^*$  value (16.5) (Table 4.5). The smaller range of  $a^*$  values over time is consistent with others who found better color stability in dark storage (Haile et al., 2013; Anderson et al., 1988). The other coolers had larger  $a^*$  ranges over time, and lower minimum  $a^*$  values (Table 4.5). Given visual discoloration was observed in coolers A-D (Appendix A.1-A.10), the

numerical data supports a loss of redness in the ham, particularly for packages near greater light intensity, which could be attributed to the formation of metmyoglobin with light as a catalyst.

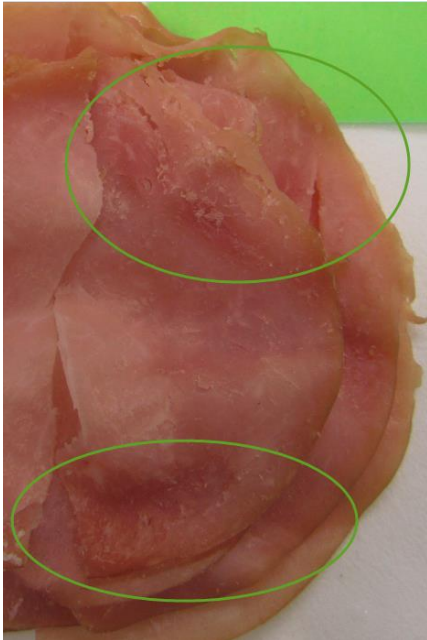
There are several other possibilities to explain the variation of ham color observed in this study beyond pH changes. A significant contributor is the initial ham color at the time of manufacture. Because the ham formulation is made of both inside and outside muscles which vary in color and contains white fat deposits (Figure 4.6 A), the starting ham color can range as much as  $\Delta a^* = 6$  (with starting values 14 – 20 within the same lot. See section 3.11). The inside ham muscle (semimembranosus), use for the Deli Express<sup>®</sup> ham, has a small muscle (gracilis) lying over the top (also is referred to as the cap in the industry). Gracilis typically has more pigment; resulting in the darker red appearance (Figure 4.6 B) The chromameter factors all of these color variations into the color calculation along with any discoloration from photo-oxidation of the meat pigment. Slices with the darker red spots will result in higher  $a^*$  values, while slices with white fat deposits will result in lower  $a^*$  calculations.



**Figure 4.6** Appearance of initial ham color at the start of sandwich assembly. A) white fat deposit B) Dark red gracilis location

Other explanations for color variations include 1) Nitrosylhemochrome can reform in the presence of residual nitrite and excess ascorbate (Ledward, Johnston, and Knight, 1992), 2) Metmyoglobin can be formed in the presence of light and low  $O_2$  (Møller et al., 2000),

3) Moisture exchanged between the meat, bread, and cheese can result in a greater concentration of the meat pigment and a more intensified color as moisture is removed from the ham (Figure 4.7). The open cooler A had the best average  $a^*$  value score (18.34), and the maximum  $a^*$  value recorded (21.44, Table 4.5), but also had observed dehydration (Figure 4.7). Cooler A being open is subject to more air flow from around the room. The concept of dehydration of the ham and intensified red color is supported by this higher average and maximum  $a^*$  as product observed in this cooler showed signs of dehydration (Figure 4.7). 4) Sheridan et al. concluded that the variation in  $a^*$  could also be attributed to the intensity of the reflected light off of the ham (resulting in photo-oxidation of the surface) (Sheridan et. al, 2007).



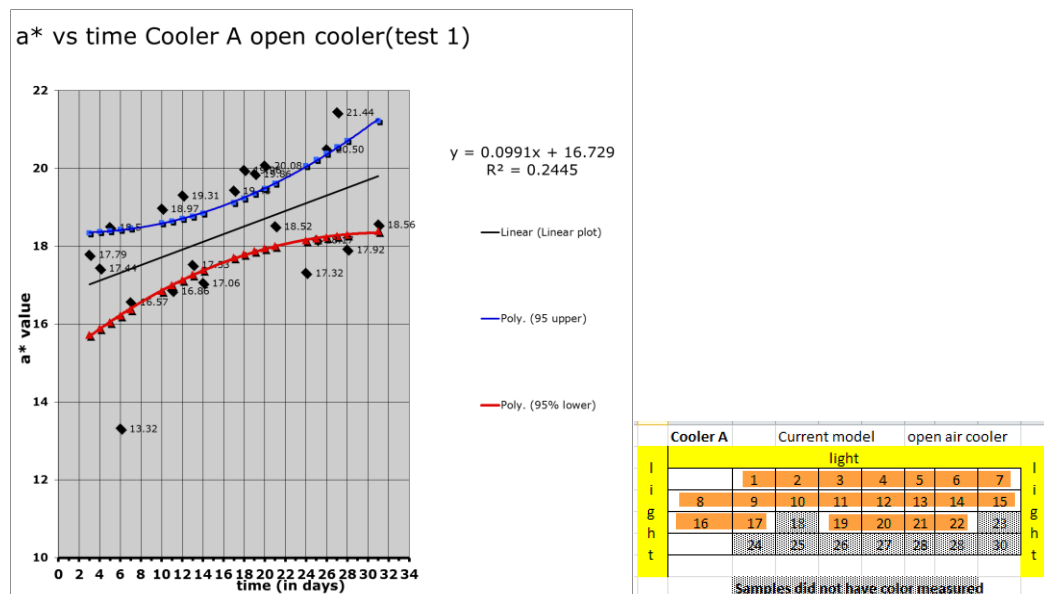
**Figure 4.7** Ham from open cooler at day 31 (areas of visible dehydration circled) Test 1

Using the statistical method outlined in section 3.20 (Zero order kinetic modeling),  $a^*$  over time was analyzed for statistical difference by comparing  $a^*$  performance over time of each cooler individually to the control cooler E (dark cooler). Three different views of this statistical comparison were considered. The first was statistically comparing all samples throughout the study regardless of cooler location. The second compared week one differences in performance, and the third compared the results of only the sandwiches

nearest the light source throughout the study (the samples nearest the light correspond to days 7, 14, 21, and 27).

Entering the  $a^*$  values from Table 4.5 above into the kinetics data input sheet (Tables 4.6 – 4.10) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

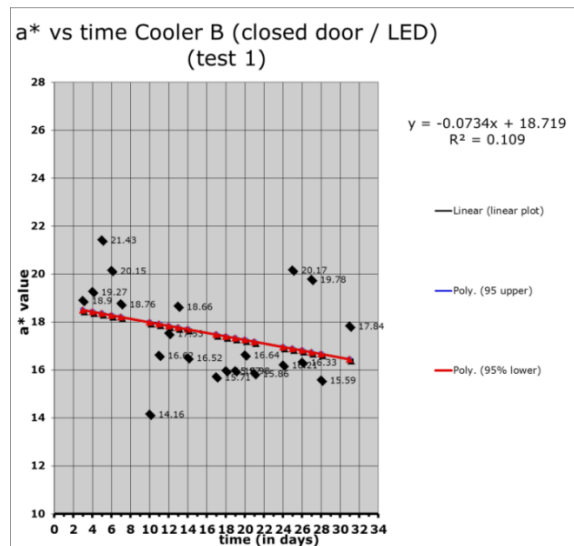
<b>1. Raw Data:</b>																		
# data pairs	Total=	21 This is automatically counted																
Y units	a*	Cooler A																
X units	days																	
<b>STATISTICS</b>																		
2. Calculations after entering Y and X you need to pull down formulas in each column from top to last entry row(yi-yes)^2										(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predictor average		
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predictor average			
17.79	3.0	316.48	17.79	17.03	9.00	17.79	17.03	0.58	175.25	53.37	9.00	18.34	15.71	2.62	17.03			
17.44	4.0	304.15	17.44	17.13	16.00	17.44	17.13	0.10	149.77	69.76	16.00	18.37	15.88	2.48	17.13			
18.5	5.0	342.25	18.50	17.22	25.00	18.50	17.22	1.63	126.29	92.50	25.00	18.40	16.05	2.35	17.22			
13.32	6.0	177.42	13.32	17.32	36.00	13.32	17.32	16.03	104.82	79.92	36.00	18.43	16.22	2.22	17.32			
16.57	7.0	274.56	16.57	17.42	49.00	16.57	17.42	0.73	85.34	115.99	49.00	18.47	16.38	2.09	17.42			
18.97	10.0	359.86	18.97	17.72	100.00	18.97	17.72	1.56	38.91	189.70	100.00	18.60	16.84	1.75	17.72			
16.86	11.0	284.26	16.86	17.82	121.00	16.86	17.82	0.92	27.44	185.46	121.00	18.65	16.99	1.66	17.82			
19.31	12.0	372.88	19.31	17.92	144.00	19.31	17.92	1.94	17.96	231.72	144.00	18.71	17.13	1.58	17.92			
17.53	13.0	307.30	17.53	18.02	169.00	17.53	18.02	0.24	10.49	227.89	169.00	18.77	17.26	1.51	18.02			
17.06	14.0	291.04	17.06	18.12	196.00	17.06	18.12	1.12	5.01	238.84	196.00	18.85	17.39	1.46	18.12			
19.43	17.0	377.52	19.43	18.41	289.00	19.43	18.41	1.03	0.58	330.31	289.00	19.12	17.71	1.42	18.41			
19.96	18.0	398.40	19.96	18.51	324.00	19.96	18.51	2.09	3.10	359.28	324.00	19.23	17.79	1.44	18.51			
19.86	19.0	394.42	19.86	18.61	361.00	19.86	18.61	1.56	7.63	377.34	361.00	19.35	17.87	1.48	18.61			
20.08	20.0	403.21	20.08	18.71	400.00	20.08	18.71	1.87	14.15	401.60	400.00	19.48	17.94	1.54	18.71			
18.52	21.0	342.99	18.52	18.81	441.00	18.52	18.81	0.08	22.68	388.92	441.00	19.62	18.00	1.62	18.81			
17.32	24.0	299.98	17.32	19.11	576.00	17.32	19.11	3.20	60.25	415.68	576.00	20.07	18.15	1.92	19.11			
18.17	25.0	330.15	18.17	19.21	625.00	18.17	19.21	1.08	76.77	454.25	625.00	20.22	18.19	2.03	19.21			
20.50	26.0	420.25	20.50	19.31	676.00	20.50	19.31	1.43	95.29	533.00	676.00	20.38	18.23	2.16	19.31			
21.44	27.0	459.67	21.44	19.41	729.00	21.44	19.41	4.14	115.82	578.88	729.00	20.55	18.26	2.29	19.41			
17.92	28.0	321.13	17.92	19.50	784.00	17.92	19.50	2.51	138.34	501.76	784.00	20.71	18.30	2.42	19.50			
18.56	31.0	344.47	18.56	19.80	961.00	18.56	19.80	1.54	217.91	575.36	961.00	21.22	18.38	2.84	19.80			
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predictor average			
															Slope=	0.0991		
															intercept=	16.7289		
															r sq=	0.2445		



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**Table 4.7** Test 1  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the closed cooler with LED lights in cooler B in all cooler lanes over 32 days

1. Raw Data:															
# data pairs	Total=	21	This is automatically counted												
Y units	a*	Cooler B LED													
X units	days														
STATISTICS															
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yes)^2 (xi-xave)^2 xi*yi X^2 y 95%UL y 95%LL Delta predicte average															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
18.9	3.0	357.21	18.90	18.50	9.00	18.90	18.50	0.16	175.25	56.70	9.00	20.08	16.92	3.16	18.50
19.27	4.0	371.33	19.27	18.43	16.00	19.27	18.43	0.71	149.77	77.08	16.00	19.92	16.93	2.99	18.43
21.43	5.0	459.24	21.43	18.35	25.00	21.43	18.35	9.47	126.29	107.15	25.00	19.77	16.94	2.83	18.35
20.15	6.0	406.02	20.15	18.28	36.00	20.15	18.28	3.50	104.82	120.90	36.00	19.61	16.94	2.67	18.28
18.76	7.0	351.94	18.76	18.21	49.00	18.76	18.21	0.31	85.34	131.32	49.00	19.46	16.95	2.52	18.21
14.16	10.0	200.51	14.16	17.99	100.00	14.16	17.99	14.63	38.91	141.60	100.00	19.04	16.93	2.11	17.99
16.62	11.0	276.22	16.62	17.91	121.00	16.62	17.91	1.67	27.44	182.82	121.00	18.91	16.91	2.00	17.91
17.53	12.0	307.30	17.53	17.84	144.00	17.53	17.84	0.10	17.96	210.36	144.00	18.79	16.89	1.90	17.84
18.66	13.0	348.20	18.66	17.77	169.00	18.66	17.77	0.80	10.49	242.58	169.00	18.67	16.86	1.82	17.77
16.52	14.0	272.91	16.52	17.69	196.00	16.52	17.69	1.37	5.01	231.28	196.00	18.57	16.81	1.76	17.69
15.71	17.0	246.80	15.71	17.47	289.00	15.71	17.47	3.10	0.58	267.07	289.00	18.32	16.62	1.70	17.47
15.97	18.0000	255.04	15.97	17.40	324.00	15.97	17.40	2.04	3.10	287.46	324.00	18.26	16.53	1.73	17.40
15.98	19.0000	255.36	15.98	17.32	361.00	15.98	17.32	1.81	7.63	303.62	361.00	18.22	16.43	1.79	17.32
16.64	20.0000	276.89	16.64	17.25	400.00	16.64	17.25	0.37	14.15	332.80	400.00	18.18	16.32	1.86	17.25
15.86	21.0000	251.54	15.86	17.18	441.00	15.86	17.18	1.74	22.68	333.06	441.00	18.15	16.20	1.95	17.18
16.21	24.0000	262.76	16.21	16.96	576.00	16.21	16.96	0.56	60.25	389.04	576.00	18.11	15.81	2.31	16.96
20.17	25.0000	406.83	20.17	16.88	625.00	20.17	16.88	10.79	76.77	504.25	625.00	18.11	15.66	2.45	16.88
16.33	26.0000	266.67	16.33	16.81	676.00	16.33	16.81	0.23	95.29	424.58	676.00	18.11	15.51	2.60	16.81
19.78	27.0000	391.25	19.78	16.74	729.00	19.78	16.74	9.25	115.82	534.06	729.00	18.11	15.36	2.75	16.74
15.59	28.0000	243.05	15.59	16.66	784.00	15.59	16.66	1.15	138.34	436.52	784.00	18.12	15.21	2.91	16.66
17.84	31.0000	318.27	17.84	16.44	961.00	17.84	16.44	1.95	217.91	553.04	961.00	18.15	14.73	3.42	16.44
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
slope= -0.0734															
intercept= 18.7193															
rsq= 0.1090															
± 95% slope 0.1006															
k upper 0.0272															
k lower -0.1740															
Standard Error 1.86															
Sum (yi-yes) 65.73															
n 21.00															
t 95% ,2 n-2= 2.09															
x average = 16.24															
Sum (xi-xav) 1493.81															
(Sum x)^2 #####															
Sum(y^2) 6525.34															
sum y 368.08															
Sum (xi*yi) 5867.29															
sum x 341.00															
sum (X^2) 7031.00															
Equations															
Y = 18.7193 -0.0734 * time															



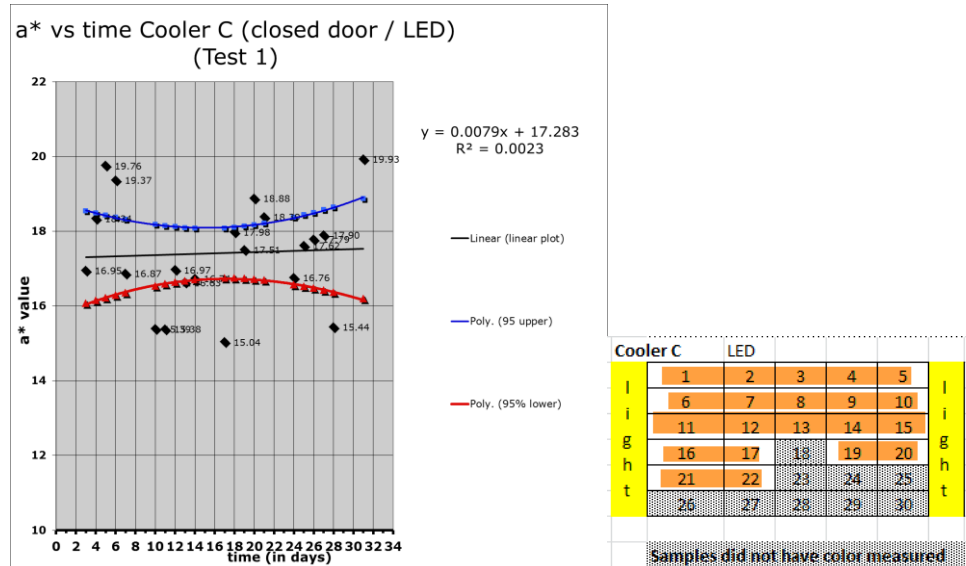
Cooler B - LED bulbs									
1	2	3	4	5					
6	7	8	9	10					
11	12	13	14	15					
16	17	18	19	20					
21	22	23	24	25					
26	27	28	29	30					

Samples did not have color measured

**Figure 4.9 A:** LED cooler (B) Test 1 Zero order Linear plot of  $a^*$  versus time (All sample numbers) with 95% confidence limits calculation B: Cooler location (highlight in orange)

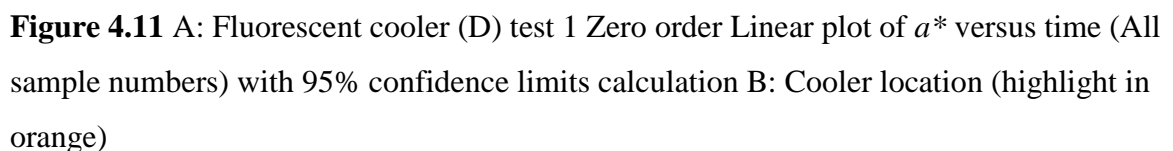
**Table 4.8** Test 1  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the closed cooler with LED lights cooler C in all cooler lanes over 32 days

1. Raw Data:															
# data pairs	Total=	21	This is automatically counted												
Y units	a*		Cooler C LED												
X units	days														
STATISTICS															
2. Calculati															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicti
16.95	3.0	287.30	16.95	17.31	9.00	16.95	17.31	0.13	175.25	50.85	9.00	18.54	16.07	2.47	17.31
18.34	4.0	336.36	18.34	17.31	16.00	18.34	17.31	1.05	149.77	73.36	16.00	18.48	16.14	2.34	17.31
19.76	5.0	390.46	19.76	17.32	25.00	19.76	17.32	5.94	126.29	98.80	25.00	18.43	16.22	2.21	17.31
19.37	6.0	375.23	19.37	17.33	36.00	19.37	17.33	4.16	104.82	116.22	36.00	18.37	16.29	2.09	17.31
16.87	7.0	284.60	16.87	17.34	49.00	16.87	17.34	0.22	85.34	118.09	49.00	18.32	16.35	1.97	17.31
15.39	10.0	236.85	15.39	17.36	100.00	15.39	17.36	3.89	38.91	153.90	100.00	18.19	16.54	1.65	17.31
15.38	11.0	236.54	15.38	17.37	121.00	15.38	17.37	3.96	27.44	169.18	121.00	18.15	16.59	1.56	17.31
16.97	12.0	287.98	16.97	17.38	144.00	16.97	17.38	0.17	17.96	203.64	144.00	18.12	16.64	1.49	17.31
16.63	13.0	276.55	16.63	17.39	169.00	16.63	17.39	0.57	10.49	216.19	169.00	18.10	16.67	1.42	17.31
16.74	14.0	280.23	16.74	17.39	196.00	16.74	17.39	0.43	5.01	234.36	196.00	18.08	16.71	1.37	17.31
15.04	17.0	226.20	15.04	17.42	289.00	15.04	17.42	5.65	0.58	255.68	289.00	18.08	16.75	1.33	17.31
17.98	18.0000	323.28	17.98	17.43	324.00	17.98	17.43	0.31	3.10	323.64	324.00	18.10	16.75	1.36	17.31
17.51	19.0000	306.65	17.51	17.43	361.00	17.51	17.43	0.01	7.63	332.69	361.00	18.13	16.73	1.40	17.31
18.88	20.0000	355.45	18.88	17.44	400.00	18.88	17.44	2.07	14.15	377.60	400.00	18.17	16.71	1.45	17.31
18.39	21.0000	338.19	18.39	17.45	441.00	18.39	17.45	0.89	22.68	386.19	441.00	18.21	16.69	1.52	17.31
16.76	24.0000	280.90	16.76	17.47	576.00	16.76	17.47	0.51	60.25	402.24	576.00	18.37	16.57	1.80	17.31
17.62	25.0000	310.46	17.62	17.48	625.00	17.62	17.48	0.02	76.77	440.50	625.00	18.44	16.52	1.91	17.31
17.79	26.0000	316.48	17.79	17.49	676.00	17.79	17.49	0.09	95.29	462.54	676.00	18.50	16.47	2.03	17.31
17.90	27.0000	320.41	17.90	17.50	729.00	17.90	17.50	0.16	115.82	483.30	729.00	18.57	16.42	2.15	17.31
15.44	28.0000	238.39	15.44	17.50	784.00	15.44	17.50	4.26	138.34	432.32	784.00	18.64	16.37	2.28	17.31
19.93	31.0000	397.20	19.93	17.53	961.00	19.93	17.53	5.77	217.91	617.83	961.00	18.87	16.19	2.68	17.31
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicti
slope= 0.0079															
intercept= 17.2829															
rsq= 0.0023															
± 95% slope 0.0787															
k upper 0.0866															
k lower -0.0708															
Standard Error 1.46															
Sum (yi-yes) 40.25															
n 21.00															
t 95%, 2,n-2 2.09															
x average= 16.24															
Sum (xi-xav) 1493.81															
(Sum x)^2 116281.00															
Sum (y^2) 6406.65															
sum y 365.64															
Sum (xi*Yi) 5949.12															
sum x 341.00															
sum (X^2) 7031.00															
Equations															
Y = 17.2829 + 0.0079 * time															



**Figure 4.10** A: LED cooler (C) test 1 Zero order Linear plot of  $a^*$  versus time (All sample numbers) with 95% confidence limits calculation B: Cooler location (highlight in orange)



[illegible]

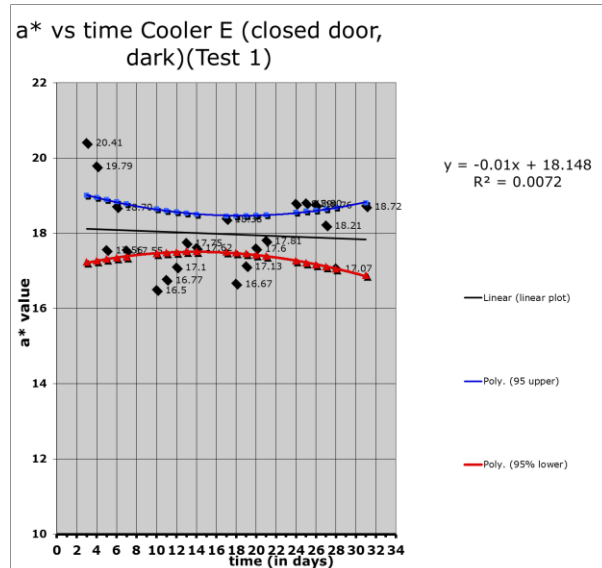
**Table 4.10** Test 1  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the closed cooler with no light cooler C in all cooler lanes over 32 days

1. Raw Data:																
# data pairs Total=		21 This is automatically counted														
Y units	a*	Cooler E	no light													
X units	days															
STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry (d yi-yes)^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	20.41	3.0	416.57	20.41	18.12	9.00	20.41	18.12	5.25	175.25	61.23	9.00	19.00	17.23	1.77	18.12
	19.79	4.0	391.64	19.79	18.11	16.00	19.79	18.11	2.83	149.77	79.16	16.00	18.95	17.27	1.68	18.11
	17.56	5.0	308.35	17.56	18.10	25.00	17.56	18.10	0.29	126.29	87.80	25.00	18.89	17.31	1.58	18.10
	18.70	6.0	349.69	18.70	18.09	36.00	18.70	18.09	0.37	104.82	112.20	36.00	18.84	17.34	1.50	18.09
	17.55	7.0	308.00	17.55	18.08	49.00	17.55	18.08	0.28	85.34	122.85	49.00	18.78	17.37	1.41	18.08
	16.5	10.0	272.25	16.50	18.05	100.00	16.50	18.05	2.40	38.91	165.00	100.00	18.64	17.46	1.18	18.05
	16.77	11.0	281.23	16.77	18.04	121.00	16.77	18.04	1.61	27.44	184.47	121.00	18.60	17.48	1.12	18.04
	17.1	12.0	292.41	17.10	18.03	144.00	17.10	18.03	0.86	17.96	205.20	144.00	18.56	17.50	1.06	18.03
	17.75	13.0	315.06	17.75	18.02	169.00	17.75	18.02	0.07	10.49	230.75	169.00	18.53	17.51	1.02	18.02
	17.62	14.0	310.46	17.62	18.01	196.00	17.62	18.01	0.15	5.01	246.68	196.00	18.50	17.52	0.98	18.01
	18.38	17.0	337.82	18.38	17.98	289.00	18.38	17.98	0.16	0.58	312.46	289.00	18.46	17.50	0.96	17.98
	16.67	18.0	277.89	16.67	17.97	324.00	16.67	17.97	1.68	3.10	300.06	324.00	18.45	17.48	0.97	17.97
	17.13	19.0	293.44	17.13	17.96	361.00	17.13	17.96	0.68	7.63	325.47	361.00	18.46	17.46	1.00	17.96
	17.6	20.0	309.76	17.60	17.95	400.00	17.60	17.95	0.12	14.15	352.00	400.00	18.47	17.43	1.04	17.95
	17.81	21.0	317.20	17.81	17.94	441.00	17.81	17.94	0.02	22.68	374.01	441.00	18.48	17.39	1.09	17.94
	18.79	24.0	353.06	18.79	17.91	576.00	18.79	17.91	0.78	60.25	450.96	576.00	18.55	17.26	1.29	17.91
	18.80	25.0	353.44	18.80	17.90	625.00	18.80	17.90	0.81	76.77	470.00	625.00	18.58	17.21	1.37	17.90
	18.76	26.0	351.94	18.76	17.89	676.00	18.76	17.89	0.76	95.29	487.76	676.00	18.61	17.16	1.45	17.89
	18.21	27.0	331.60	18.21	17.88	729.00	18.21	17.88	0.11	115.82	491.67	729.00	18.65	17.11	1.54	17.88
	17.07	28.0	291.38	17.07	17.87	784.00	17.07	17.87	0.64	138.34	477.96	784.00	18.68	17.05	1.63	17.87
	18.72	31.0	350.44	18.72	17.84	961.00	18.72	17.84	0.78	217.91	580.32	961.00	18.80	16.88	1.92	17.84
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	Equations															
	Y = 18.1478 -0.0100 * time															

slope=	-0.0100
intercept=	18.1478
rsq=	0.0072
± 95% slope	0.0564
k upper	0.0464
k lower	-0.0664

Equations

$$Y = 18.1478 - 0.0100 * \text{time}$$



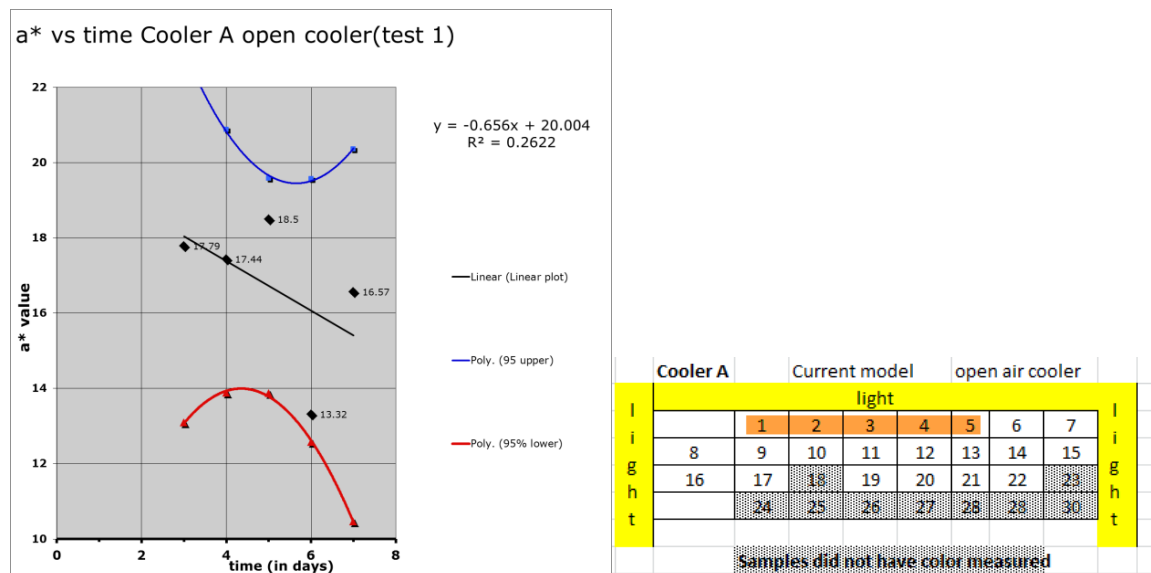
Cooler E					- no light				
1	2	3	4	5					
6	7	8	9	10					
11	12	13	14	15					
16	17	18	19	20					
21	22	23	24	25					
26	27	28	29	30					

Samples did not have color measured

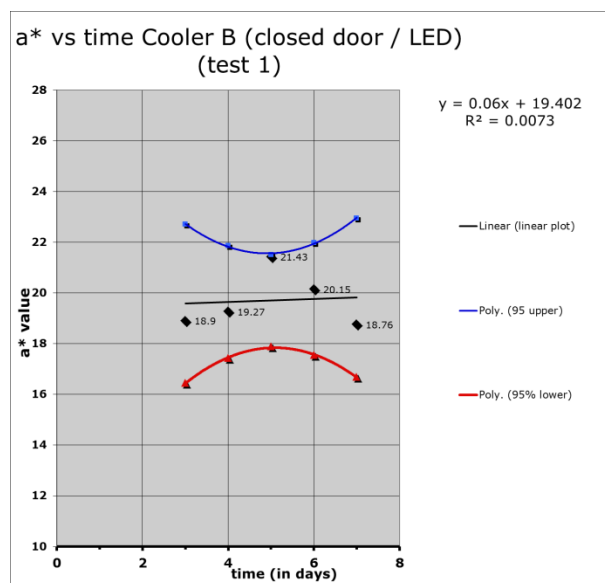
**Figure 4.12 A:** Dark cooler (E) test 1 Zero order Linear plot of  $a^*$  versus time (All sample numbers) with 95% confidence limits calculation B: Cooler location (highlighted in orange)

The outcome of  $a^*$  value variability and low  $R^2$  values in evaluating all samples is not unexpected given the complexity of this food system described above, and the number of reactions taking place over time.

Because of the variability over 30 days resulting in poor  $R^2$  values (Figures 4.8 – 4.12), the results were also evaluated in the chemical kinetics model over a short period of time (1 week) (Tables 4.11-4.15). However, the statistical comparison of all coolers for one week (day 3-7) did not improve all  $R^2$  values in cooler A-D ( $R^2 = 0.0072 - 0.2622$ , Figure 4.13 – 4.16), but the  $R^2$  for cooler E was improved ( $R^2 = 0.694$ , Figure 4.17) showing a decrease in  $a^*$  values over time, which indicates light exposure and low residual oxygen are not the only factors affecting the color of the ham. The trend line for the  $a^*$  slope in coolers A, D, and E indicate a negative slope (less red as indicated by a decrease in  $a^*$  value), while the LED coolers indicate a slight improvement in red (increasing  $a^*$  values). This poor fit of data can be attributed to the high day to day variability of  $a^*$  values in each sample. The  $\Delta a^*$  within each cooler ranged as much as 6.26 (fluorescent bulb cooler D) to little as 2.67 (LED bulb cooler B) over 1 week. The position of each sample was a horizontal line on the cooler shelf with different distances from the light source.

[illegible]

112

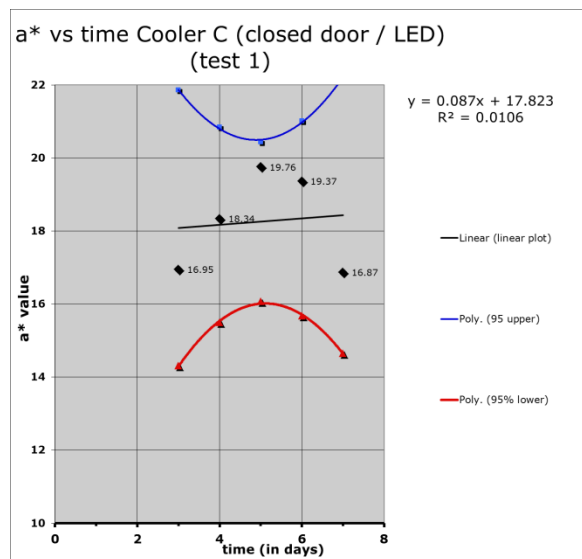
[illegible]

Cooler B - LED bulbs					
	1	2	3	4	5
l i g h t	6	7	8	9	10
	11	12	13	14	15
	16	17	18	19	20
	21	22	23	24	25
	26	27	28	29	30
	Samples did not have color measured				

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**Table 4.13** Test 1  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the LED cooler (C) in all cooler lanes over 7 days

1. Raw Data:																
# data pairs Total=			5 This is automatically counted													
Y units			a*													
X units			days													
STATISTICS																
2. Calculate: Note after entering Y and X you need to pull down formulas in each column from top to last entry $(y_i - y_{est})^2$																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate		$(x_i - x_{ave})^2$	$x_i * y_i$	X^2	y 95%UL	y 95%LL	Delta	predicted average
	16.95	3.0	287.30	16.95	18.08	9.00	16.95	18.08	1.29	4.00	50.85	9.00	21.86	14.31	7.56	18.08
	18.34	4.0	336.36	18.34	18.17	16.00	18.34	18.17	0.03	1.00	73.36	16.00	20.84	15.50	5.34	18.17
	19.76	5.0	390.46	19.76	18.26	25.00	19.76	18.26	2.26	0.00	98.80	25.00	20.44	16.08	4.36	18.26
	19.37	6.0	375.20	19.37	18.35	36.00	19.37	18.35	1.05	1.00	116.22	36.00	21.02	15.67	5.34	18.35
	16.87	7.0	284.60	16.87	18.43	49.00	16.87	18.43	2.44	4.00	118.09	49.00	22.21	14.65	7.56	18.43
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	$(y_i - y_{est})^2$	$(x_i - x_{ave})^2$	$X_i * Y_i$	X^2	y 95%UL	y 95%LL	Delta	predicted average
	slope=				0.0870				Standard Error				1.53			
	intercept=				17.8230				Sum (yi-yes)				1913.02			
	rsq=				0.0106				n				5.00			
	± 95% slope				1.5428				t 95% 2, n-2=				3.18			
	k upper				1.6298				x average =				5.00			
	k lower				-1.4558											
	Equations				Y = 17.8230 + 0.0870 * time				Sum (xi-xav)				160.00			
									(Sum x)^2				625.00			
									Sum(y^2)				1673.91			
									sum y				91.29			
									Sum (xi*yi)				457.32			
									sum x				25.00			
									sum (X^2)				135.00			

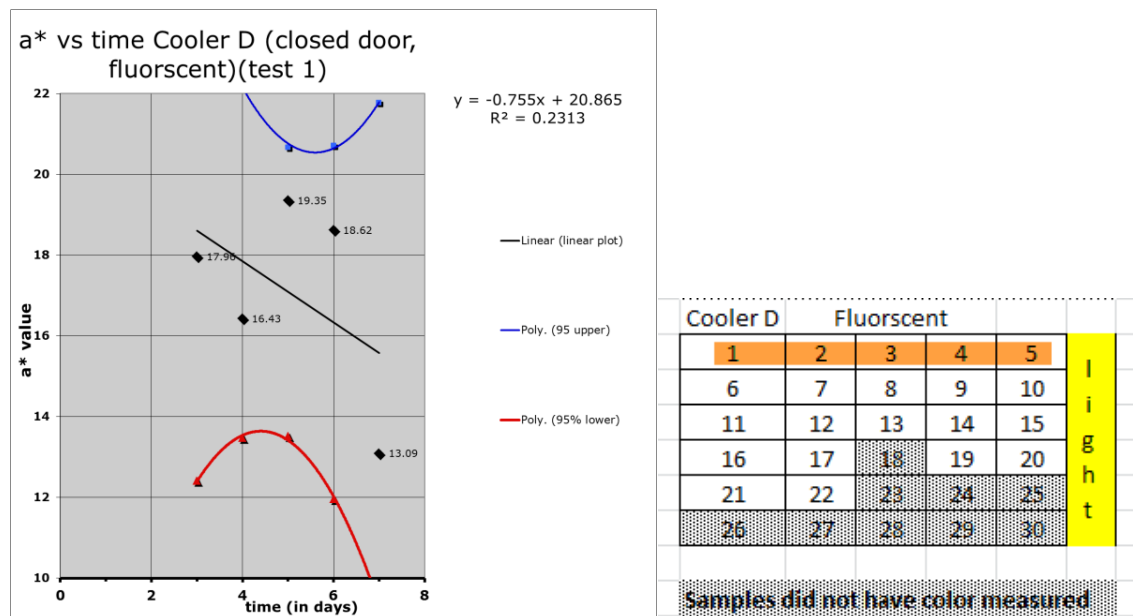


Samples did not have color measured						
Cooler C		LED				
l i g h t	1	2	3	4	5	l i g h t
	6	7	8	9	10	
	11	12	13	14	15	
	16	17	18	19	20	
	21	22	23	24	25	
	26	27	28	29	30	

**Figure 4.15 A:** LED cooler (C) Test 1 Zero order Linear plot of  $a^*$  versus time (the first week – sample numbers 1-5) with 95% confidence limits calculation B: Cooler location (highlighted in orange)

**Table 4.14** Test 1  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the fluorescent cooler (D) in all cooler lanes over 7 days

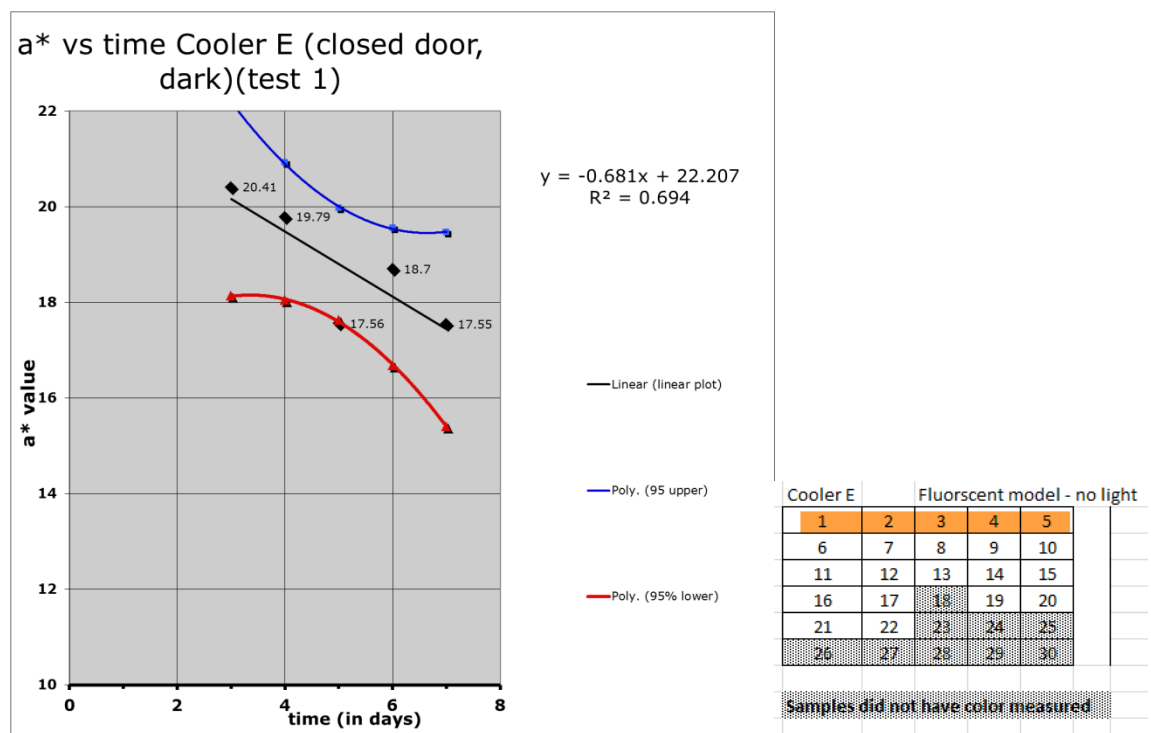
# data pairs	Total=	5	This is automatically counted														
Y units	a'																
X units	days																
STATISTICS																	
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry (d(yi-yes)^2)																	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate		(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	
	17.96	3.0	322.56	17.96	18.60	9.00	17.96	18.60	0.41	4.00	53.88	9.00	24.79	12.41	12.38	18.60	
	16.43	4.0	269.94	16.43	17.85	16.00	16.43	17.85	2.00	1.00	66.72	16.00	22.22	13.47	8.75	17.85	
	19.35	5.0	374.42	19.35	17.09	25.00	19.35	17.09	5.11	0.00	96.75	25.00	20.66	13.52	7.15	17.09	
	18.62	6.0	346.70	18.62	16.34	36.00	18.62	16.34	5.22	1.00	111.72	36.00	20.71	11.96	8.75	16.34	
	13.09	7.0	171.35	13.09	15.58	49.00	13.09	15.58	6.20	4.00	91.63	49.00	21.77	9.39	12.38	15.58	
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	



**Figure 4.16** A: Fluorescent cooler (D) Test 1 Zero order Linear plot of  $a^*$  versus time (the first week – sample numbers 1-5) with 95% confidence limits calculation B: Cooler location (highlighted in orange)

**Table 4.15** Test 1  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the dark cooler (E) in all cooler lanes over 7 days

1. Raw Data:																	
# data pairs Total=		5 This is automatically counted															
Y units		a*															
X units		days															
STATISTICS																	
2. Calculate: Note after entering Y and X you need to pull down formulas in each column from top to last entry $r(yi-yes)^2$																	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate		$(xi-xave)^2$	$xi*yi$	X^2	y 95%UL	y 95%LL	Delta	predicted average	
	20.41	3.0	416.57	20.41	20.16	9.00	20.41	20.16	0.06	4.00	61.23	9.00	22.20	18.13	4.07	20.16	
	19.79	4.0	391.64	19.79	19.48	16.00	19.79	19.48	0.09	1.00	79.16	16.00	20.92	18.05	2.88	19.48	
	17.56	5.0	308.35	17.56	18.80	25.00	17.56	18.80	1.54	0.00	87.80	25.00	19.98	17.63	2.35	18.80	
	18.7	6.0	349.69	18.70	18.12	36.00	18.70	18.12	0.34	1.00	112.20	36.00	19.56	16.68	2.88	18.12	
	17.55	7.0	308.00	17.55	17.44	49.00	17.55	17.44	0.01	4.00	122.85	49.00	19.47	15.41	4.07	17.44	
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	$(yi-yes)^2$	$(xi-xave)^2$	$Xi*Yi$	X^2	y 95%UL	y 95%LL	Delta	predicted average	
	slope=				-0.6810					Standard Error				0.83			
	intercept=				22.2070					Sum (yi-yes)				8385.61			
	rsq=				0.6940					n				5.00			
	± 95% slope				0.83002					t 95%, 2, n-2=				3.18			
	k upper				0.1492					x average =				5.00			
	k lower				-1.5112												
	Equations				Y = 22.2070 - 0.6810 * time					Sum (xi-xav)				435.00			
										$(\sum x)^2$				625.00			
										$\sum (y^2)$				1774.26			
										sum y				94.01			
										$\sum (xi*yi)$				463.24			
										sum x				25.00			
										$\sum (X^2)$				135.00			

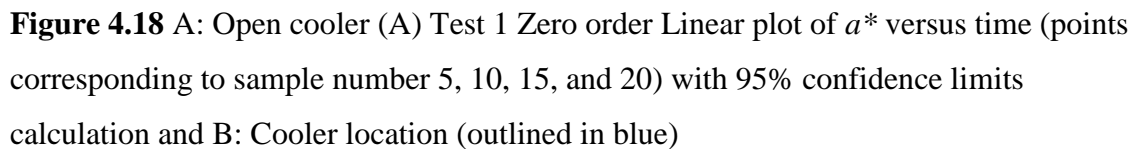


**Figure 4.17** A: Dark cooler (E) Test 1 Zero order Linear plot of  $a^*$  versus time (the first week – sample numbers 1-5) with 95% confidence limits calculation and B: Cooler location (highlighted in orange)

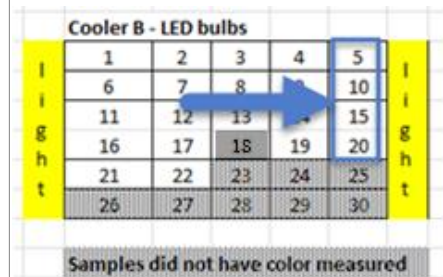


Due to the observation of the lowest  $a^*$  values for each of coolers A – D occurring closest to the light source, a third approach was to compare statistical performance per cooler of those samples that were nearest the light source (this correlated to days 7, 14, 21 and 27 of the shelf life). The results of the kinetics rate constant calculations are found in Tables 4.16 – 4.20.

In cooler B (with LED light), the day 7, 14, and 21 samples decrease in  $a^*$  value, but it increase at day 27 (Table 4.17). Explanations of why are discussed above as likely a change in mechanism. As a result of the increased value at day 27, the calculated  $R^2$  for this cooler is poor (Figure 4.19). In cooler C, the trend line shows increasing  $a^*$  values over time (Figure 4.20). The fluorescent cooler D consistently shows low  $a^*$  values at days 7, 14, and 21, followed by an increase at day 27 (Figure 4.21) as with cooler B. The dark cooler E shows an improvement over time, and a tight range of predicted slopes at the 95% confidence level (Figure 4.22). For cooler A, the increasing  $a^*$  over time (Figure 4.18) could be attributed to the ham dehydration as described above. The air flow over the open cooler could create a driving force to remove moisture from the package even with the low Water Vapor Transmission Rate (WVTR) of the film (Section 3.3 & 3.4) Because the configuration of this cooler was the most unique, the samples from this cooler also did not align with being nearest the light source (Table 4.2). This could also explain the higher  $a^*$  values as they were further from the light source.

[illegible]

1. Raw Data:																
# data pairs Total=	4	This is automatically counted														
Y units	a*															
X units	days															
STATISTICS																
2. Calculation: Note after entering Y and X you need to pull down formulas in each column from top to last entry (y1-yes)*2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(y1-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%LL	Delta	predicte average
	18.76	7.0	351.94	18.76	17.44	49.00	18.76	17.44	1.74	105.06	131.32	49.00	25.60	9.28	16.33	17.44
	16.52	14.0	272.91	16.52	17.64	196.00	16.52	17.64	1.25	10.56	231.28	196.00	22.89	12.39	10.50	17.64
	15.86	21.0	251.54	15.86	17.84	441.00	15.86	17.84	3.90	14.06	333.06	441.00	23.22	12.45	10.78	17.84
	19.78	27.0	391.25	19.78	18.01	729.00	19.78	18.01	3.15	95.06	534.06	729.00	25.91	10.10	15.81	18.01
			0.00	0.00	17.24	0.00	0.00	17.24	297.31	297.56	0.00	0.00	29.33	5.15	24.18	17.24
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(y1-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%LL	Delta	predicte average
					slope=		0.0283							Standard Er	2.24	
					intercept=		17.2426							Sum (y1-yes)	2091.20	
					rsq=		0.0175							n	4.00	
					± 95% slope		0.6428							t 95%, 2, n-2=	4.30	
					k upper		0.6711							x average =	17.25	
					k lower		-0.6146									
														Sum (xi-xav)	2307.69	
														(Sum x)^2	4761.00	
														Sum(y^2)	1267.64	
														sum y	70.92	
														Sum (xi*yi)	1229.72	
														sum x	69.00	
														sum (X^2)	1415.00	
					Equations											
					Y =	17.2426	0.0283	* time								

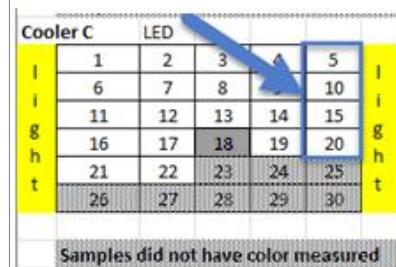
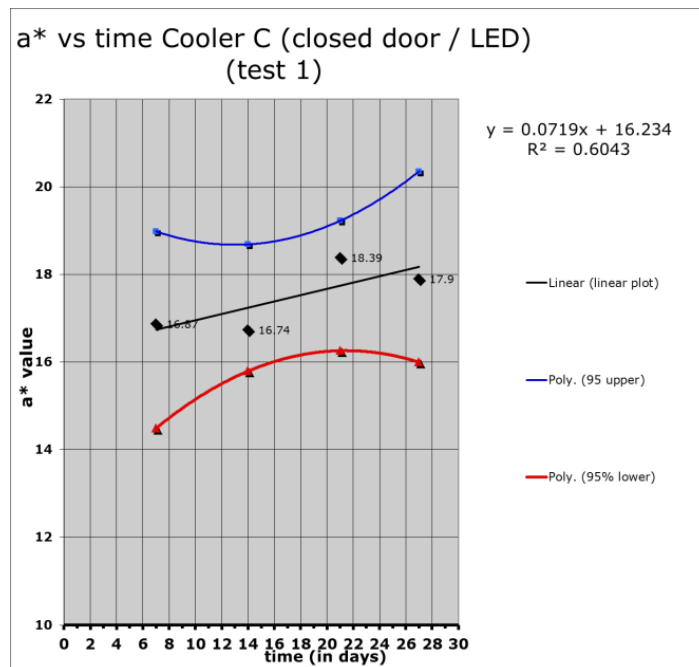


119

<b>1. Raw Data:</b>	
# data pairs Total=	4 This is automatically counted
Y units	a*
X units	days

										STATISTICS						
2. Calculati: Note after entering Y and X you need to pull down formulas in each column from top to last entry r(yi-yes)^2										(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%LL	Delta	predicte average
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2								
16.87	7.0	284.60	16.87	16.74	49.00	16.87	16.74	0.02	105.06	118.09	49.00	18.99	14.49	4.49	16.74	
16.74	14.0	280.23	16.74	17.24	196.00	16.74	17.24	0.25	10.56	234.36	196.00	18.69	15.80	2.89	17.74	
18.39	21.0	338.19	18.39	17.74	441.00	18.39	17.74	0.42	14.06	386.19	441.00	19.23	16.26	2.97	17.74	
17.9	27.0	320.41	17.90	18.18	729.00	17.90	18.18	0.08	95.06	483.30	729.00	20.35	16.00	4.35	18.18	
		0.00	0.00	16.23	0.00	0.00	16.23	263.55	297.56	0.00	0.00	19.56	12.91	6.66	16.23	
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%JL	y 95%LL	Delta	predicte average	
slope=				0.0719								Standard Er		0.62		
intercept=				16.2343								Sum (yi-yes)		1845.63		
rsq=				0.6043								n		4.00		
± 95% slope				0.1770								t 95%, 2,n-2=		4.30		
k upper				0.2489								x average =		17.25		
k lower				-0.1051												
Equations																
Y =		16.2343		0.0719		* time										
										Sum (xi-xav		2307.69				
										(Sum x)^2		4761.00				
										Sum(y^2)		1223.43				
										sum y		69.90				
										Sum (xi*yi)		1221.94				
										sum x		69.00				
										sum (X^2)		1415.00				

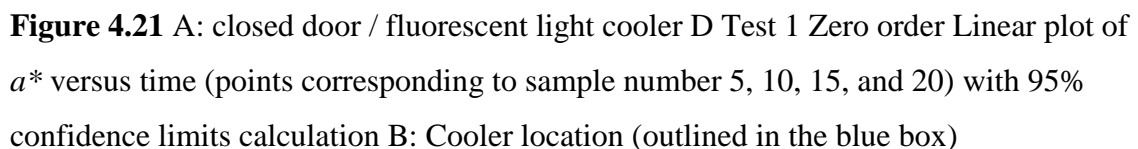


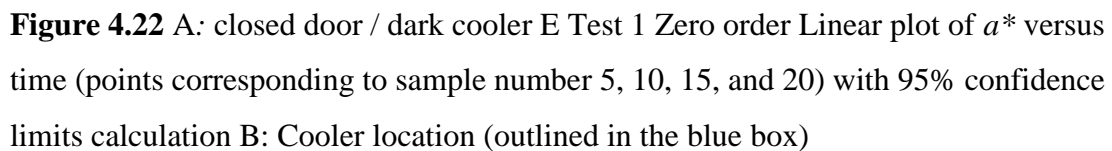
120

<b>1. Raw Data:</b>	
# data pairs Total=	4 This is automatically counted
Y units	a*
X units	days

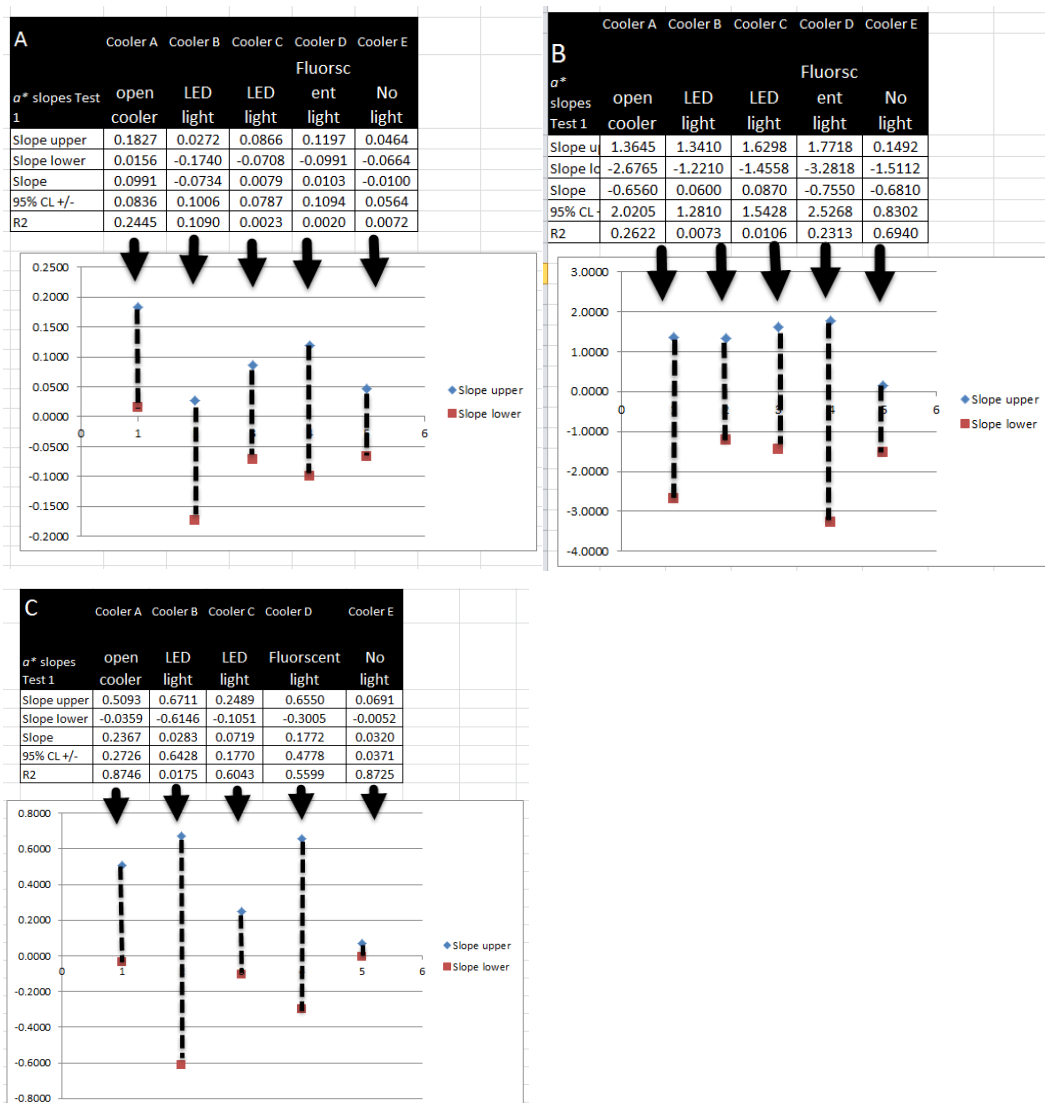
										STATISTICS						
<b>2. Calculation:</b> Note after entering Y and X you need to pull down formulas in each column from top to last entry $(y_i - \text{yes})^2$										$(x_i - \text{xave})^2$	$x_i * y_i$	$X^2$	y 95%JUL	y 95%LL	Delta	predicte average
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate								
	13.09	7.0	171.35	13.09	12.31	49.00	13.09	12.31	0.61	105.06	91.63	49.00	18.38	6.24	12.13	12.31
	13.14	14.0	172.66	13.14	13.55	196.00	13.14	13.55	0.17	10.56	183.96	196.00	17.45	9.65	7.81	13.55
	13.07	21.0	170.82	13.07	14.79	441.00	13.07	14.79	2.96	14.06	274.47	441.00	18.79	10.79	8.01	14.79
	17.2	27.0	295.84	17.20	15.85	729.00	17.20	15.85	1.81	95.06	464.40	729.00	21.73	9.98	11.75	15.85
			0.00	0.00	11.07	0.00	0.00	11.07	122.49	297.56	0.00	0.00	20.05	2.08	17.97	11.07
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	$(y_i - \text{yes})^2$	$(x_i - \text{xave})^2$	$X_i * Y_i$	$X^2$	y 95%JUL	y 95%LL	Delta	predicte average
	slope=				0.1772				Standard Error				1.67			
	intercept=				11.0676				Sum (yi-yes)				862.99			
	rsq=				0.5599				n				4.00			
	± 95% slope				0.4778				t 95%, 2,n-2=				4.30			
	k upper				0.6550				x average =				17.25			
	k lower				-0.3005											
	Equations															
	Y = 11.0676				0.1772		* time									



[illegible]

Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments viewed over 32 days, 7 days and nearest the light is provided in Table 4.21. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 4.21** Test 1  $a^*$  rate constant ( $k$ ) upper and lower for all applications as established by Labuza' Reaction kinetics shelf life model. A: 32 day shelf life. B: days 3-7. C: For Lane A (near the light source)



While comparing the difference of the performance of the samples nearest the light source did not result in statistical differences, it did demonstrate better color stability for product stored in the dark under similar conditions (Table 4.21). Statistically the model predicts similar performance of the cured ham in open and closed door coolers with LED and fluorescent bulbs.

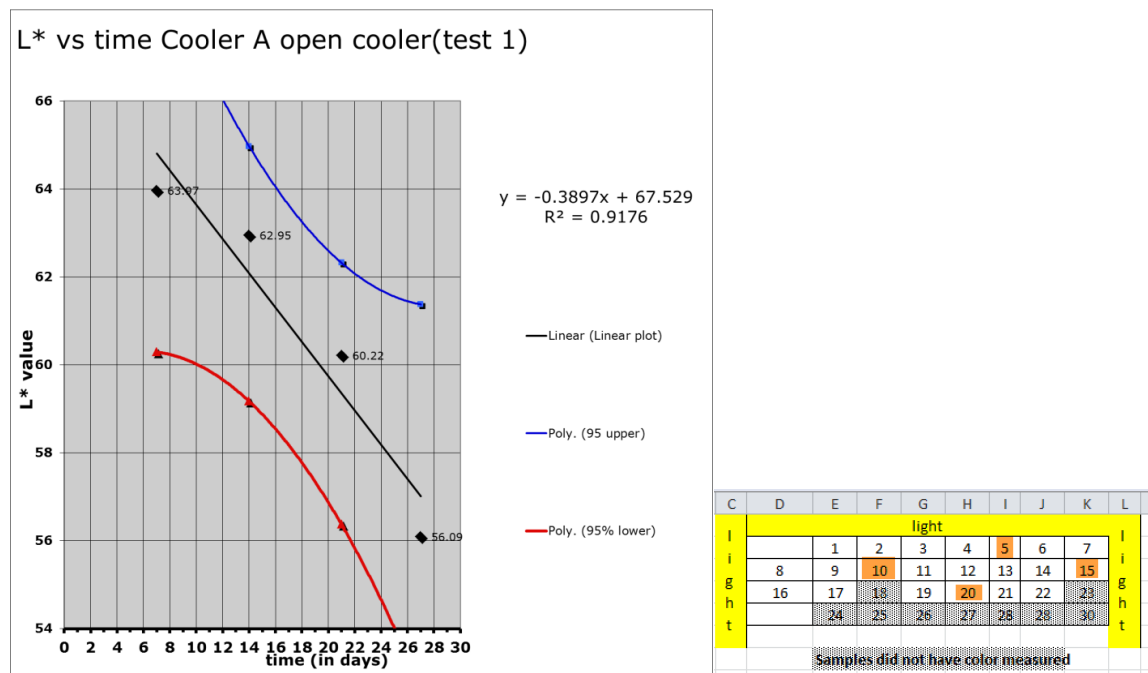
#### **4.1.5 $L^*$ values**

Using this third approach of comparing performance of samples nearest the light for  $L^*$  performance also yields additional insights into color changes. Unlike  $a^*$  values, the  $R^2$  values reveal better fit of data and more consistency over time (Figures 4.23 – 4.27). The trend for all coolers is a decreasing  $L^*$  value (darkening) over time. This could be the result of the formation of brown metmyoglobin causing a darkening of the ham. Cooler E shows a more gradual decrease over time suggesting some color changes occur for reasons other than light, but indicating light accelerates these changes in coolers A – D. Anderson et al. found Hunter  $a$  to provide a better correlation with subjective color score than Hunter  $L$  or  $b$  values (Anderson et al., 1988) Li et al. focused on  $a^*$  values and did not report  $L^*$  values (Li et al., 2012). Yen et al. reported observed differences in  $L$  values between treatments of salami, but concluded the difference in visual lightness in the product was small and would not be noticeable to consumers (Yen et al., 1988) A challenge in interpreting  $L^*$  value is increases in  $L^*$  could indicate fading and development in lighter grey, however as the red layer of pigment thins and discolorations emerge, a darkening of the overall sample can also occur.



**Table 4.22** Test 1  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the open cooler (A) samples the nearest the light source

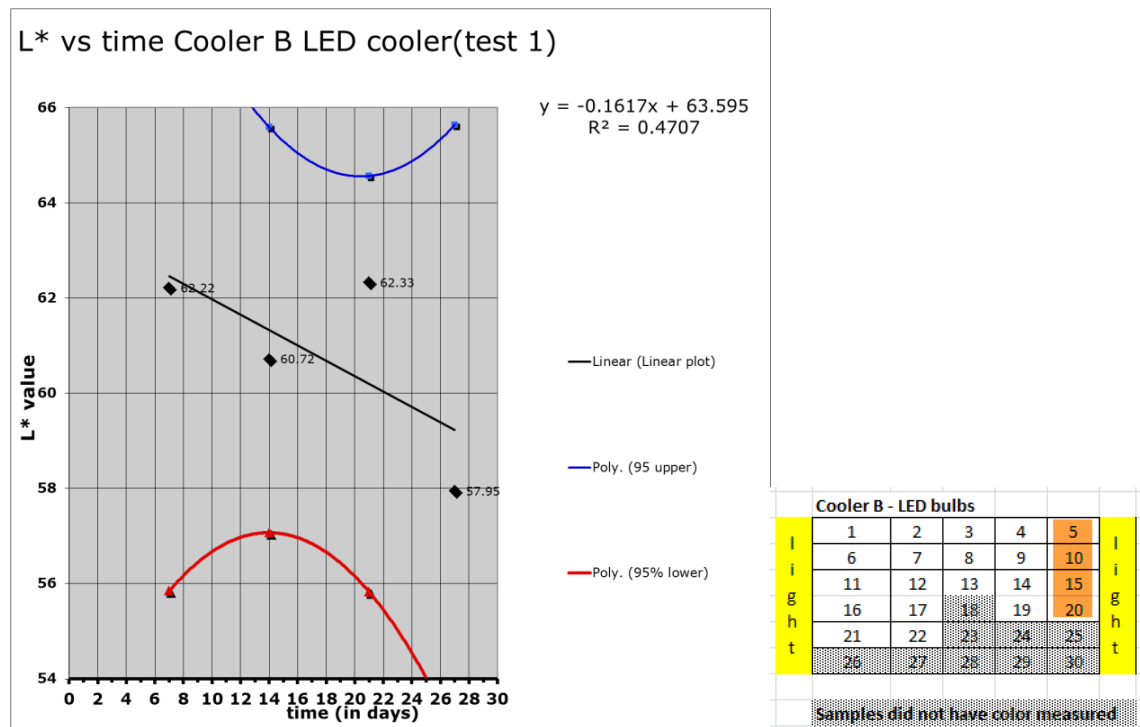
1. Raw Data:																			
# data pairs Total=		4	This is automatically counted																
Y units		a*																	
X units		days																	
										STATISTICS									
2. Calculate Note after entering Y and X you need to pull down formulas in each column from top to last entry $r(yi-yes)^2$																			
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate		$(xi-xave)^2$	$xi*yi$	X^2	y 95%UL	y 95%LL	Delta	predicte average			
	63.97	7.0	4092.16	63.97	64.80	49.00	63.97	64.80	0.69	105.06	447.79	49.00	69.31	60.29	9.02	64.80			
	62.95	14.0	3962.70	62.95	62.07	196.00	62.95	62.07	0.77	10.56	881.30	196.00	64.98	59.17	5.80	62.07			
	60.22	21.0	3626.45	60.22	59.35	441.00	60.22	59.35	0.76	14.06	1264.62	441.00	62.32	56.37	5.95	59.35			
	56.09	27.0	3146.09	56.09	57.01	729.00	56.09	57.01	0.84	95.06	1514.43	729.00	61.38	52.64	8.73	57.01			
			0.00	0.00	67.53	0.00	0.00	67.53	4560.20	297.56	0.00	0.00	74.21	60.85	13.36	67.53			
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	$(yi-yes)^2$	$(xi-xave)^2$	$Xi*Yi$	X^2	y 95%UL	y 95%LL	Delta	predicte average			



**Figure 4.23** A: Open cooler (A) Test 1 Zero order Linear plot of  $L^*$  versus time (points corresponding to sample number 5, 10, 15, and 20) with 95% confidence limits calculation B: Cooler location (highlighted in orange)

**Table 4.23** Test 1  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the closed cooler (B) with LED lights samples the nearest the light source

1. Raw Data:															
# data pairs Total=		4	This is automatically counted												
Y units		a*													
X units		days													
STATISTICS															
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry (dyi-yes)*^2															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	
62.22	7.0	3871.33	62.22	62.46	49.00	62.22	62.46	0.06	105.06	435.54	49.00	69.08	55.84	13.24	62.46
60.72	14.0	3686.92	60.72	61.33	196.00	60.72	61.33	0.37	105.56	850.08	196.00	65.59	57.07	8.52	61.33
62.33	21.0	3885.03	62.33	60.20	441.00	62.33	60.20	4.54	14.06	1308.93	441.00	64.57	55.83	8.74	60.20
57.95	27.0	3358.20	57.95	59.23	729.00	57.95	59.23	1.63	95.06	1564.65	729.00	65.64	52.82	12.82	59.23
		0.00	0.00	63.59	0.00	0.00	63.59	4044.27	297.56	0.00	0.00	73.40	53.79	19.61	63.59
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
												Standard Error	1.82		
												Sum (yi-yes)	#####		
												n	4.00		
												t 95% ,2,n-2=	4.30		
												x average =	17.25		
												Sum (xi-xav)	2307.69		
												(Sum x)^2	4761.00		
												Sum(y^2)	#####		
												sum y	243.22		
												Sum (xi*yi)	4159.20		
												sum x	69.00		
												sum (X^2)	1415.00		
Equations															
Y = 63.5945 -0.1617 * time															



**Figure 4.24** A: LED light cooler (B) Test 1 Zero order Linear plot of  $L^*$  versus time (points corresponding to sample number 5, 10, 15, and 20) with 95% confidence limits calculation B: Cooler location (highlighted in orange)

<b>1. Raw Data:</b>					
# data pairs Total=	4 This is automatically counted				
Y units	a*				
X units	days				

STATISTICS

2. Calculati Note after entering Y and X you need to pull down formulas in each column from top to last entry r(yi-yes)^2									(xi-xave)^2	x^2yi	X^2	y 95%JL	y 95%LL	Delta	predicte average
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate								
62.76	7.0	3838.82	62.76	62.06	49.00	62.76	62.06	0.49	105.06	439.32	49.00	66.51	57.61	8.90	62.06
59.35	14.0	3522.42	59.35	60.76	196.00	59.35	60.76	1.98	10.56	830.90	196.00	63.62	57.89	5.72	60.76
60.17	21.0	3620.43	60.17	59.45	441.00	60.17	59.45	0.52	14.06	1263.57	441.00	62.39	56.51	5.87	59.45
58.32	27.0	3401.22	58.32	58.33	729.00	58.32	58.33	0.00	95.06	1574.64	729.00	62.64	54.02	8.62	58.33
		0.00	0.00	63.37	0.00	0.00	63.37	4015.43	297.56	0.00	0.00	69.96	56.78	13.18	63.37
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	X^2Yi	X^2	y 95%JL	y 95%LL	Delta	predicte average

slope=	-0.1865
intercept=	63.3674
r sq=	0.7239
+ 95% slope	0.3503
k upper	0.1637
k lower	-0.5368

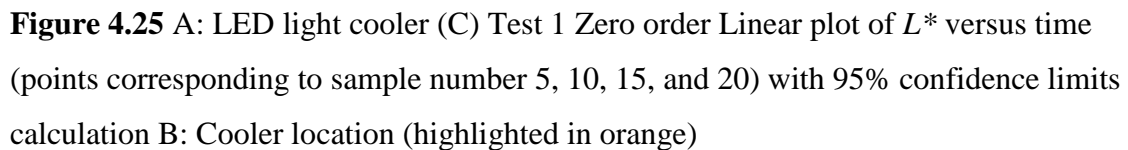
  

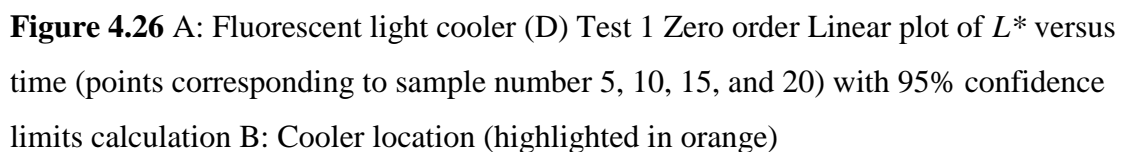
**Equations**

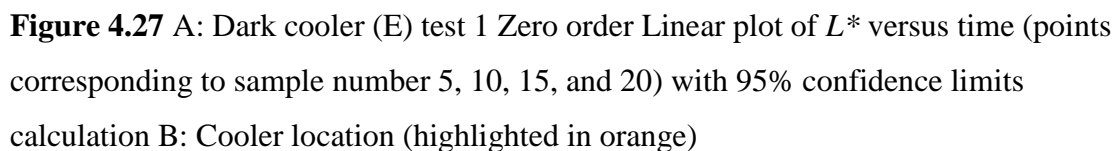
Y = 63.3674	-0.1865	* time
-------------	---------	--------

Standard Er	1.22
Sum (yi-yes)	#####
n	4.00
t 95%, 2,n-2=	4.30
x average =	17.25
Sum (xi-xav)	2307.69
(Sum x)^2	4761.00
Sum(y^2)	#####
sum y	240.60
Sum (x^iyi)	4108.43
sum x	69.00
Sum (X^2)	1415.00

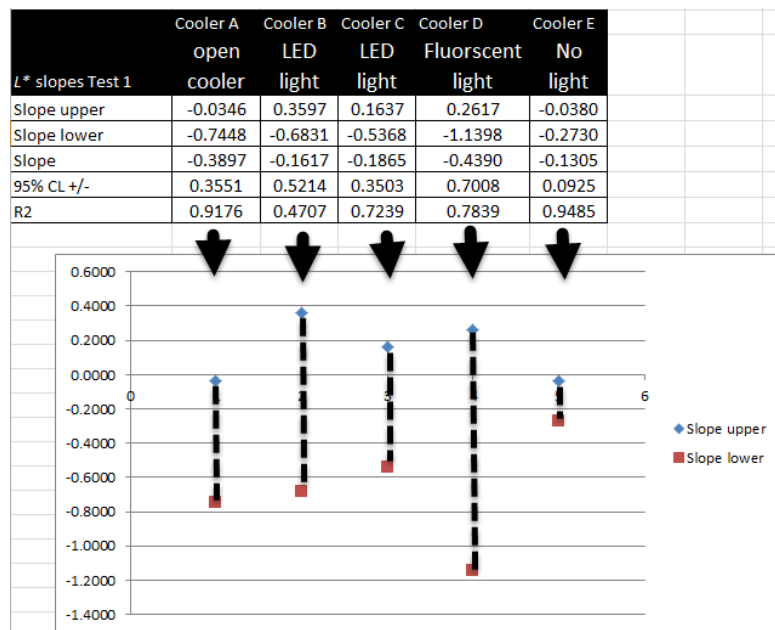


[illegible]

[illegible]

Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges ( $+k$  for lightening over the shelf life,  $-k$  for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.27.

**Table 4.27**  $L^*$  parameter rate constant ( $k$ ) upper and lower for all applications in Test 1 all lanes as established by Labuza' Reaction kinetics shelf life model.



When comparing all five coolers for  $L^*$  performance over time, all are not statistically different from each other as the range of predicted values have areas of overlap for possible scores at the 95% confidence level.

#### 4.1.6 Visual appearance ham – Test 1

The outcome of this investigation was to identify if a practical solution to cooler type and bulb management (that prevents or slows visual detection of cured ham discoloration during the established 30 day refrigerated shelf life for EAS) exists. If any of the samples develop visual grey or brown colors (regardless of  $L^*$  and  $a^*$  numerical data) during the

course of the shelf life or have a significantly faded appearance (meaning the pink color is lighter or washed out on the surface), the test solution is ineffective (in this test LED bulbs with no UV emissions). The chromameter was used to numerically interpret pink color ( $a^*$  value) and fading ( $L^*$ ). For Test 1, visual observations were made on unwrapped ham at days 5, 6, 11, 13, 14, 19, 20, 21, 26 and 31.

It is well established that color of the meat influences consumer purchase intent (Sindelar et al., 2007), however there is no research available establishing a specific level of discoloration as a marker for when consumers reject the product, other than complete loss of redness was established as unacceptable (which will be reviewed in chapter 5 in the FPI / Deli Express<sup>®</sup> study, 2014) Because the photooxidation reaction is complex and not fully understood, it is difficult to create representative samples. For the purpose of this study, any visual detection of loss of redness is viewed as negative. In the visual comparisons, cooler E (dark cooler) serves as the control for the other coolers to be compared to. A comparison can also be made within the same sandwich by comparing the area under the label to the exposed area. (See example in Appendix A.11) In this test, visual discoloration was observed throughout the study in all lighted coolers (Appendix A.1 – A.10).

#### **4.1.6 Cooler temperatures Test 1**

The cooler temperatures varied over time (raw data in A.13). Comparing the three closed door coolers (B, C, D, and E), the warmest average cooler C (3.68 C°) did not have the lowest averaged  $a^*$  value. The coldest cooler E (0.57 C°) did have the highest average  $a^*$  score of the closed door coolers (17.99). (Table 4.5 above) Display cabinet temperature can affect color life. (Hunt et al., 2012) The recommended display case temperatures are between 0° to 2° C for non-abuse display research (Hunt et al., 2012). Many retailers report coolers at abuse temperatures. However for sliced cured ham, cooler temperature has proven to not be a significant factor in color outcome (Nannerup et al., 2004). Further evidence of cooler temperature not being a significant factor can be seen with cooler D having the lowest  $a^*$  values (13.07, Table 4.5), despite having the lowest temperature of the coolers with lights (0.88 C°, Table 4.28). Case temperature is not the same as product temperature, but is an important factor to report (Hunt et al., 2012). The

open cooler did prove to be the warmest cooler, but did not result in greater discolored samples.

**Table 4.28** Average cooler temperatures Test 1

cooler	average temp C°
Cooler A	5.91
Cooler B	3.03
Cooler C	3.68
Cooler D	0.88
Cooler E	0.57

#### 4.1.8 Conclusions Test 1

Statistically, there was no difference in  $a^*$  value performance over time for any of the cooler/light combinations evaluated. The range of predicted possible slopes in each cooler would have overlapped as established by zero order reaction kinetics spreadsheets. (Table 4.21, Table 4.27) The most predictable outcomes (as measured by  $R^2$ ) and smallest range of predicted slopes at the 95% confidence level was in cooler E (dark cooler), which reinforces the influence of light.  $L^*$  value analysis indicates a darkening (decreasing  $L^*$ ) in all coolers, even the dark cooler E, suggesting that other factors impact color changes, and photooxidation isn't the only process responsible for color change. While there is not a statistical difference between coolers on  $a^*$  or  $L^*$  values, there are trends and visual evidence to make key observations and areas for future focus. Proximity to the light appears to accelerate discoloration. Directionally this test established that the minimum  $a^*$  scores all occurred on sandwiches nearest the light source (Figure 4.5 for  $a^*$  scores, Table 4.2 for positions), with the exception of the darkened cooler E. Greater discoloration in close proximity of light is in agreement with Sheridan et al. findings (Sheridan et al., 2007)



Dark storage in this study resulted in less color variability, but ham still undergoes color changes as demonstrated by the variability of  $a^*$  in the dark cooler E (Figure 4.22) and  $L^*$  values over time (Figure 4.27). The complexity of meat pigment formation, coupled with the dynamic environment created with the other heterogeneous sandwich components, MAP process, and extended refrigerated shelf life help provide insight into potential causes of the variability of the color scores.

Use of LED lights did not prevent visual discoloration. Visual discoloration and fading developed in all coolers except cooler E (dark). (Appendix A.1 – A.10) This outcome is in agreement with the Møller et al. observation that cured meats are nearly equally affected by both UV and visible light for discoloration development (Møller et al., 2000). These results are unlike results obtained with fresh meat. Fresh pork loins retained a more desirable color under Grolux wide spectrum bulbs compared to cool white fluorescent (Kopf, Hung and Hunt, 1987), and fresh beef in MAP under lighting without UV radiation which resulted in a significant delay in spoilage as measured by surface color (Djenane et al., 2001). This may be explained by differences between raw meat pigments and cooked cured meat pigment. This is in agreement with Skibsted who found that the light wavelength dependence for discoloration of fresh meats is more significant than cured meats (Skibsted, 1992).

## **4.2a Test 2a - Impact of varied oxygen levels in the packaging headspace through equipment adjustments**

### **4.2.1a Overview of Test 2a**

The goal of this test was to assess the impact of various oxygen levels in the package headspace (0, 0.5 and 5.0 %) for the development of meat discoloration. In this test, attempts were made to create a Modified Atmosphere Package (MAP) with 0% and 5.0% residual oxygen in the headspace by adjusting the gas flush equipment settings, to compare to the current target of <0.5% O<sub>2</sub>. If oxygen can be successfully eliminated from the package, a necessary component of the photo-oxidation reaction is removed and meat discoloration could be prevented. Because photo-oxidation of nitrosylmyoglobin has been found to be linearly dependent on the amount of oxygen present (Møller, Bertelsen, and Skibsted, 2002), the predicted outcome of a 5.0% targeted headspace oxygen product should show a significant increase in the development of discoloration as compared to a package with less than 0.5%.

### **4.2.2a Methods**

Two Beverage Air coolers (Model # LV27 c) with fluorescent bulbs were used in this study. (This is the same model number as Cooler D and E from Test 1) Each cooler was set up to hold one test variable and a control sample for comparison. Cooler A held three rows of control samples (at 0.5% oxygen headspace target) and three rows of the test sample at a target of 0% oxygen in the headspace. Cooler B held three rows of control samples (at 0.5% oxygen headspace target) and three rows of the test sample (at 5.0% oxygen headspace target). The sandwiches were placed one sandwich deep on the shelf. Each cooler contained a total of 40 sandwiches (20 control and 20 test samples) (Table 4.29).

**Table 4.29** Test 2a cooler set up. Samples labeled C represent the control product (0.5% oxygen in the headspace). Cooler A samples shaded in green represent the 5.0% O<sub>2</sub> headspace samples. Cooler B samples shaded in blue represent the 0% O<sub>2</sub> headspace samples.

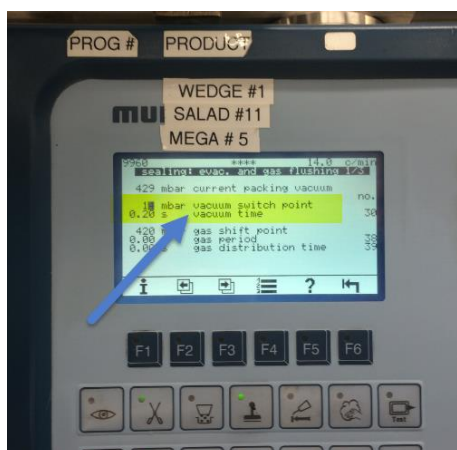
Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

The product was evaluated thirteen times throughout the twenty nine days refrigerated shelf life (a total of 26 of the 40 sandwiches in each cooler). Table 4.30 indicates the sample number evaluated and corresponding day in shelf life.

**Table 4.30** Test 2a Sample number evaluation and corresponding day in shelf life. Grey shaded areas represent days not reviewed

Day	Date	Evaluation	Sandwiches for Color	Control # pulled	5% # pulled	0% # pulled
Day 0	3/8/2012		sandwiches not reviewed			
Day 1	3/9/2012	1	1	27, 7,	6	6
Day 2	3/10/2012		sandwiches not reviewed			
Day 3	3/11/2012		sandwiches not reviewed			
Day 4	3/12/2012	2	2	26, 6,	5	5
Day 5	3/13/2012		sandwiches not reviewed			
Day 6	3/14/2012	3	3	25, 5,	4	4
Day 7	3/15/2012		sandwiches not reviewed			
Day 8	3/16/2012	4	4	24, 4,	3	3
Day 9	3/17/2012		sandwiches not reviewed			
Day 10	3/18/2012		sandwiches not reviewed			
Day 11	3/19/2012	5	5	23, 3,	14	14
Day 12	3/20/2012		sandwiches not reviewed			
Day 13	3/21/2012	6	6	34, 14	13	13
Day 14	3/22/2012		sandwiches not reviewed			
Day 15	3/23/2012	7	7	33, 13	12	12
Day 16	3/24/2012		sandwiches not reviewed			
Day 17	3/25/2012		sandwiches not reviewed			
Day 18	3/26/2012	8	8	32, 12	11	11
Day 19	3/27/2012		sandwiches not reviewed			
Day 20	3/28/2012	9	9	31, 11	10	10
Day 21	3/29/2012		sandwiches not reviewed			
Day 22	3/30/2012	10	10	30, 10	20	20
Day 23	3/31/2012		sandwiches not reviewed			
Day 24	4/1/2012		sandwiches not reviewed			
Day 25	4/2/2012	11	11	39, 19	19	19
Day 26	4/3/2012		sandwiches not reviewed			
Day 27	4/4/2012	12	12	38, 18	18	18
Day 28	4/5/2012		sandwiches not reviewed			
Day 29	4/6/2012	13	13	37, 17	17	17

Equipment adjustments to the Multivac included reducing the vacuum switch point (decreasing from standard 14 millibar setting to 1 millibar) and changing the length of vacuum time (increasing the amount of evacuation time from 0.2 seconds to 0.3 seconds) (Figure 4.28).



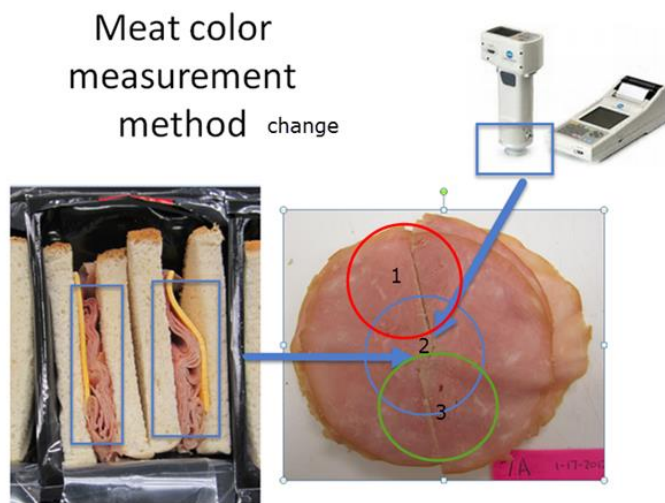
**Figure 4.28** Multivac equipment setting changes required for influencing the amount of oxygen evacuated from a package

The assembled packaged sandwiches spent approximately 12 days in dark frozen storage, in a corrugated case, before refrigerated shelf life began. On each evaluation day, one control and one test variable were removed from each cooler and evaluated for headspace oxygen,  $L^*$  and  $a^*$  color and visual inspection. Compared sandwich locations in this study were offset by one position due to leaving a sample in place near the light throughout the study as a photo reference (Table 4.31 – samples left in place for photos labeled as “pic”) See Table 4.31 for an example of offset sampling (numbers circled in red represent the products compared within each cooler).

**Table 4.31** Example of offset sampling. On day 6, the control sample 34 was pulled and evaluated with the test variable sample number 13

Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

All samples were checked for  $O_2$  using a Mocon analyzer (as described in section 3.12), and measured for color using a Minolta Chroma Meter (as described in section 3.10). Sandwiches in this study were measured for  $L^*$  and  $a^*$  values using method 1 (Reconstructing and flattening “bunched” ham into a flat surface and measuring the center where the two half slices meet). For each ham measurement in Test 2, a change was made for measuring color. Instead of a single surface measurement, three different positions on the surface of the ham were measured and averaged to represent single  $L^*$  and  $a^*$  values per AMSA recommendations (Hunt et al., 2012) (Figure 4.29). Raw data for  $L^*a^*b^*$  calculations for both Tests 2a and 2b are located in Appendix B.17.



**Figure 4.29** Chromameter measurement area on ham surface. The colored circles labeled 1, 2, and 3 represent the locations of the measurements that were averaged to create a single  $L^*$  and  $a^*$  value to represent the sample

Ham color (once removed from the package) was also visually inspected at each color measurement day (documented in Appendix B.2 – B.8). Photos were taken to document visual color (Method in section 3.10).

Each sandwich component used was taken from the same production lot to minimize batch to batch variability. The ham and cheese was stored at approximately  $0^{\circ}\text{C}$  prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly. The bread was stored at room temperature (approximately  $21^{\circ}\text{C}$ ) prior to assembly. The

length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). All sandwiches were produced and pulled from the same sandwich production lot to minimize differences in the materials used.

#### 4.2.3a Oxygen percentage in the package headspace - Test 2

For this test, the control samples averaged significantly less than 0.5% oxygen, the 5.0% targeted average was close to desired settings (averaging 5.63 %), while the 0% average attempt failed (achieving an average of 0.51% oxygen in the headspace) (Table 4.32) The control samples on average and at maximum values were within the range stated to be critical for oxygen (0.1 to 0.5%) by Møller et. al (Møller, Weber, and Bertelsen, 1999). Both test samples (0%, 5.0%) were outside of the critical range which should result in visual discoloration. See Appendix B.1 for raw data on measured O<sub>2</sub> and CO<sub>2</sub>.

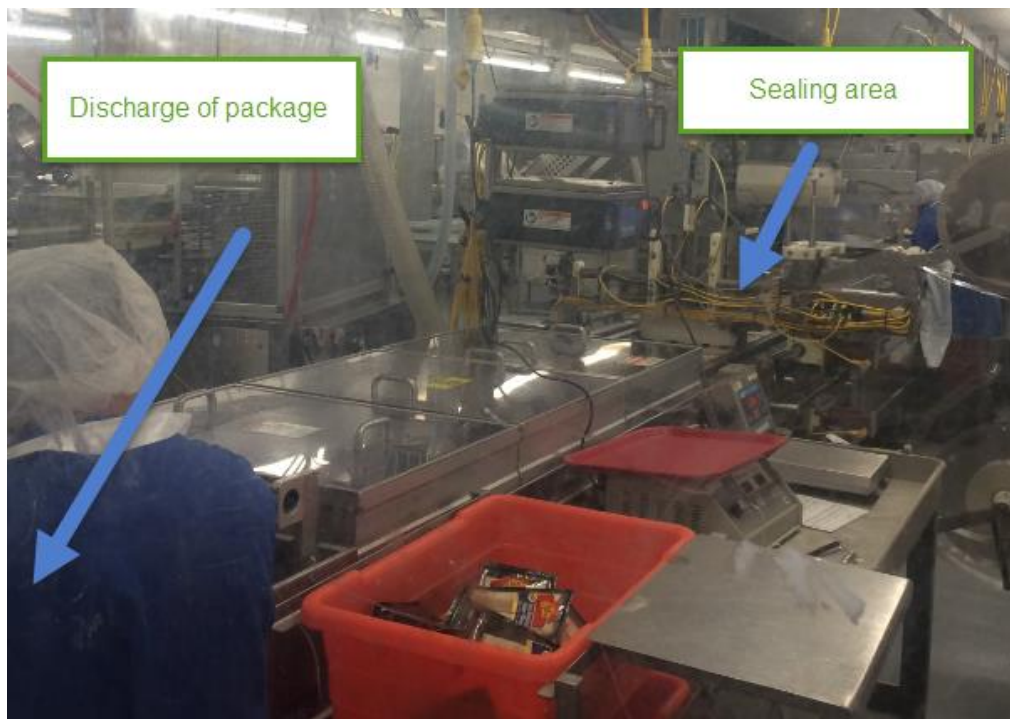
**Table 4.32** Oxygen headspace content per package – average, minimum and maximum – Test 2

Item	Average % O <sub>2</sub>	Min % O <sub>2</sub>	Max % O <sub>2</sub>
Test targeting 0% O <sub>2</sub>	0.51	0	1.91
Test targeting 5% O <sub>2</sub>	5.63	2.57	15
Control (0.5% O <sub>2</sub> target) for 0% comparison	0.04	0	0.16
Control (0.5% O <sub>2</sub> target) for 5% comparison	0.05	0.01	0.10

This testing confirmed that adjusting MAP equipment is not a viable method to remove all residual oxygen. A change in equipment settings with a short amount of run time yielded erratic results. Even when the targeted 5.0 % average was close to being met, the range was too broad to be of use (O<sub>2</sub> = 2.57% – 15% Table 4.32)

Factors that need to be considered when adjusting equipment settings for removing more oxygen are 1) purging the packaging in the pipeline, 2) barometric pressure on the day of testing, and 3) The right combination of evacuation time for the desired strength of

vacuum. Approximately 150 sandwiches are present from the point of evacuation to where the packaging is trimmed into individual units (Figure 4.30). Attempts between adjustments should be separated and the proper amount of packages removed to avoid mixing of samples. Barometric pressure changes day to day (ranging from 960 to 990 millibar at this manufacturing site). The higher the barometric pressure, the longer the evacuation time required to achieve desired levels. Making the appropriate equipment adjustments is accomplished by trial and error. In a live production environment as was the case for these sandwiches, often the time isn't available for significant trial and error. Longer run times leads to more consistent results as adjustments can be made over time. Because the environment is not static, each day explains another area for possible variation in headspace oxygen from package to package.

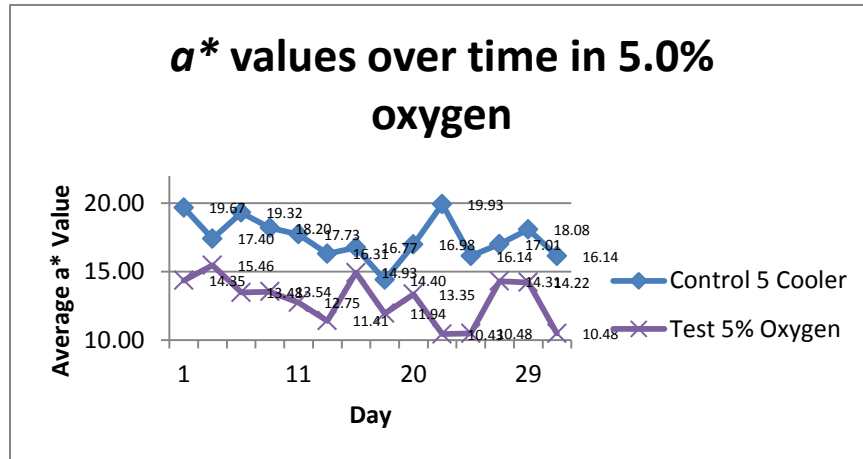


**Figure 4.30** Multivac layouts, evacuation chamber location in relation to the discharge area of packages. The distance between arrows holds approximately 150 sandwiches



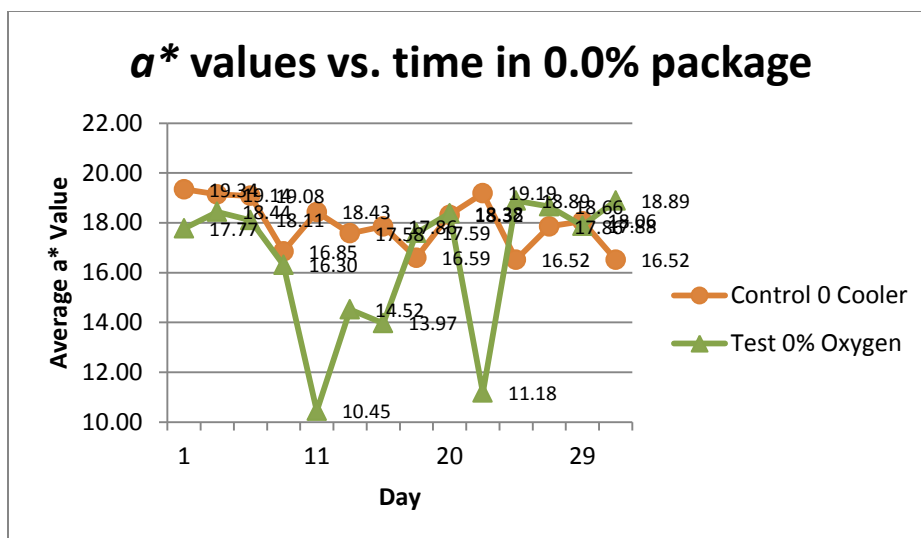
#### 4.2.4a - $a^*$ analysis Test 2a

The greatest difference in  $a^*$  value on a point by point basis was observed between the 5.0% O<sub>2</sub> package and control package (<0.5% O<sub>2</sub>), with the 5.0% samples consistently lower in  $a^*$  value compared to the >0.5% package (Figure 4.31).



**Figure 4.31**  $a^*$  values over time for the control sample compared to sample packaged in 5.0 % oxygen (achieved through machine settings) – Test 2a

The  $a^*$  values for the attempted 0% packages (through machine adjustments) at different points in the shelf life were less compared to the control, but not consistently as some of the  $a^*$  values in the attempted 0% exceed the control (Figure 4.32). This outcome can be understood when considering some of the attempted 0% samples exceeded 0.5% (Appendix B.1 for raw data).



**Figure 4.32**  $a^*$  values over time for the control sample compared to sample packaged in 0 % oxygen (achieved through machine settings) – Test 2a

On a point by point basis, the  $\Delta a^*$  was greater than 4 on 7 of 13 days evaluated, with the 5.0% product with a lower  $a^*$  value (less redness) (Table 4.33).

**Table 4.33** -  $\Delta a^*$  difference (control  $a^*$  (in yellow) minus 5.0%  $O_2$   $a^*$  (in grey)) Test 2

day	Control package (>0.5% $O_2$ )			5% $O_2$ package			$\Delta a^*$ ( $a^*$ control - $a^*$ 5%)
	control sample $a^*$	$O_2$ %		sample	$a^*$	$O_2$ %	
1	Control 5.27	19.67	0.08	Test 5 #6	14.35	5.19	5.32
4	Control 5.26	17.40	0.07	Test 5 #5	15.46	4.50	1.94
6	Control 5.25	19.32	not measured	Test 5 #4	13.48	not measured	5.83
8	Control 5.24	18.20	0.10	Test 5 #3	13.54	15.00	4.66
11	Control 5.23	17.73	0.06	Test 5 #14	12.75	8.38	4.98
13	Control 5.34	16.31	0.01	Test 5 #13	11.41	6.92	4.89
15	Control 5.33	16.77	0.05	Test 5 #12	14.93	2.57	1.83
18	Control 5.32	14.40	0.02	Test 5 #11	11.94	3.00	2.45
20	Control 5.31	16.98	0.08	Test 5 #10	13.35	4.95	3.63
22	Control 5.30	19.93	0.06	Test 5 #20	10.43	3.37	9.50
25	Control 5.39	16.14	0.01	Test 5 #19	10.48	4.53	5.66
27	Control 5.38	17.01	0.07	Test 5 #18	14.31	4.68	2.69
29	Control 5.37	18.08	0.05	Test 5 #17	14.22	5.00	3.86

Directionally, others have found that a  $\Delta a^*$  of 4 points correlates to visual differences detectable by a sensory color panel (Anderson and Rasmussen, 1992). Given the high oxygen values for the 0% attempted samples, this test was not useful other than to

confirm that it is difficult to attain 0% through equipment settings, and that the lowest  $a^*$  values for this group of samples was achieved nearest the light source. The lowest score in  $a^*$  value for the control (14.4) was at day 18, which corresponded to sandwiches located in lane C on the cooler (Tables 4.30 for sample number, Table 4.31 for location). For the 5.0% O<sub>2</sub> package, the lowest recorded  $a^*$  value (10.43) was on day 22 and located in lane B (Tables 4.30 for sample number, Table 4.31 for location). Entering the  $a^*$  values from Table 4.33 above into the kinetics data input sheet (Tables 4.134 – 4.135) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

<b>1. Raw Data:</b>					
# data pairs Total=	13	This is automatically counted			
Y units	a*				
X units	days				

STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rct(yi-yes)*2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	14.35	1	205.92	14.35	14.03	1.00	14.35	14.03	0.10	204.71	14.35	1.00	15.90	12.16	3.74	14.03
	15.46	4	239.01	15.46	13.84	16.00	15.46	13.84	2.63	127.86	61.84	16.00	15.43	12.24	3.19	13.84
	13.48	6	181.71	13.48	13.71	36.00	13.48	13.71	0.05	86.63	80.88	36.00	15.14	12.29	2.85	13.71
	13.54	8	183.33	13.54	13.59	64.00	13.54	13.59	0.00	53.40	108.32	64.00	14.86	12.32	2.54	13.59
	12.75	11	162.56	12.75	13.40	121.00	12.75	13.40	0.42	18.56	140.25	121.00	14.48	12.31	2.17	13.40
	11.41	13	130.19	11.41	13.27	169.00	11.41	13.27	3.47	5.33	148.33	169.00	14.28	12.27	2.01	13.27
	14.93	15	222.90	14.93	13.15	225.00	14.93	13.15	3.18	0.09	223.95	225.00	14.12	12.17	1.95	13.15
	11.94	18	142.56	11.94	12.96	324.00	11.94	12.96	1.04	7.25	214.92	324.00	13.98	11.94	2.04	12.96
	13.35	20	178.22	13.35	12.83	400.00	13.35	12.83	0.27	22.02	267.00	400.00	13.94	11.73	2.21	12.83
	10.43	22	108.78	10.43	12.71	484.00	10.43	12.71	5.18	44.79	229.46	484.00	13.93	11.48	2.45	12.71
	10.48	25	109.83	10.48	12.52	625.00	10.48	12.52	4.15	93.94	262.00	625.00	13.97	11.06	2.91	12.52
	14.31	27	204.78	14.31	12.39	729.00	14.31	12.39	3.68	136.71	386.37	729.00	14.02	10.76	3.26	12.39
	14.22	29	202.21	14.22	12.27	841.00	14.22	12.27	3.82	187.48	412.38	841.00	14.08	10.45	3.62	12.27
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average

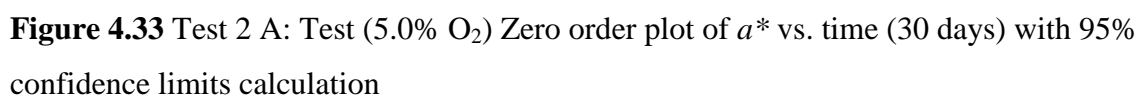
slope=	-0.0629
intercept=	14.0900
rsq=	0.1227
± 95% slope	0.1116
k upper	0.0487
k lower	-0.1745

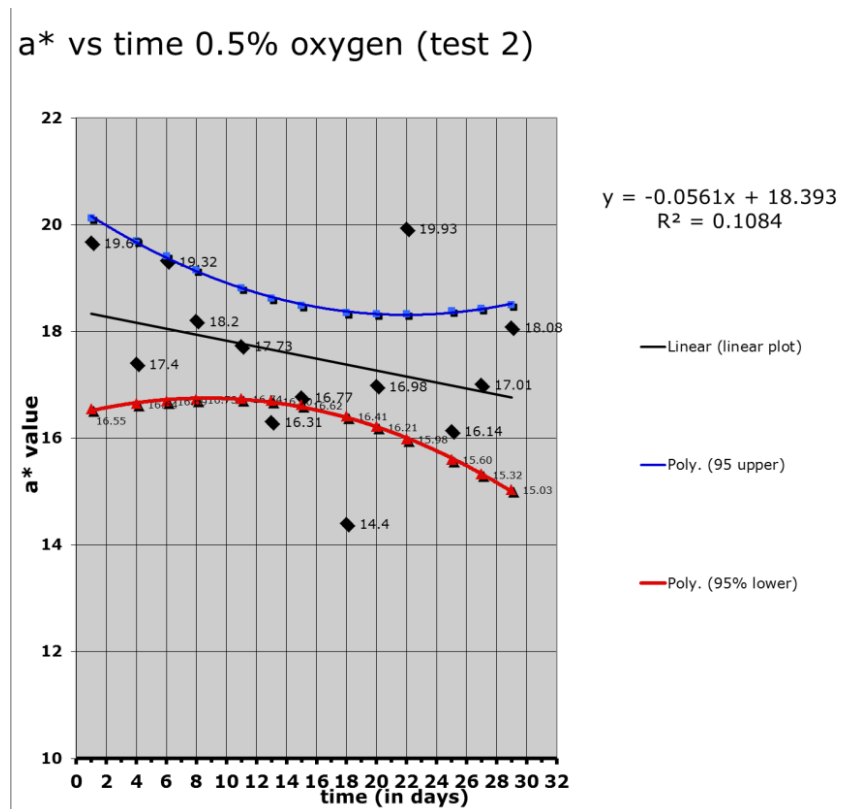
  

<b>Equations</b>	
Y =	14.0900 -0.0629 * time

Standard Error	1.60
Sum (yi-yes)	27.99
n	13.00
t 95%,2,n-2=	2.20
x average =	15.31
Sum (xi-xav	988.77
(Sum x)^2	####
Sum(y^2)	2272.02
sum y	170.65
Sum (xi*yi)	2550.05
sum x	199.00
sum (X^2)	4035.00

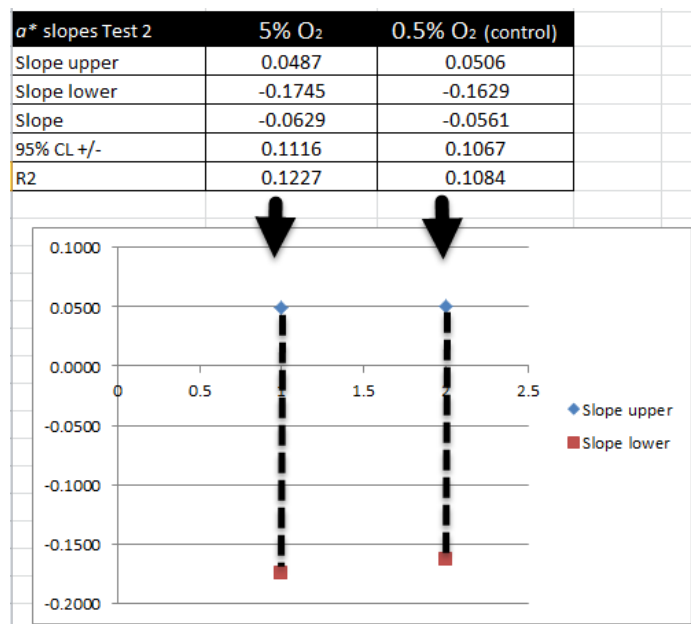


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Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments is provided in Table 4.36. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 4.36** Test 2  $a^*$  rate constant ( $k$ ) upper and lower for all applications as established by Labuza' Reaction kinetics shelf life model



When compared to the control, the 5.0% residual oxygen package is not statistically different at the 95% confidence level on  $a^*$  values (Table 4.36). The line of best fit for predicted  $a^*$  value color scores over time for the 5.0% O<sub>2</sub> sample was decreasing (meaning a loss of redness) from approx.  $a^* = 13.5$  to  $a^* = 12$ . (Figure 4.33), while the line of best fit for the control (targeted 0.5% O<sub>2</sub>) was also decreasing from approximately  $a^* = 18.2$  to  $a^* = 16.5$ . (Figure 4.34) The  $R^2$  values however are poor indicating a poor fit of data. This poor fit of data can be attributed to the variability in starting color for each individual piece of ham and other factors discussed in Test 1. As predicted based on

0.5% residual oxygen per package being a critical value to color stability, (Møller et al. 1999) the packages with the highest oxygen levels (5.0%) scored lowest in  $a^*$  values. Moges Haile et al. study on cooked ham found that better color stability is observed when MAP packages have less residual oxygen, but not based on  $a^*$  value parameters. Comparing two MAP packages, one with a 50% gas back (gas back is the amount of gas volume that is put back after vacuum is applied) of a 30% CO<sub>2</sub> / 70% N<sub>2</sub> blend and 80% vacuum (higher oxygen) to 75% gas back of a 30% CO<sub>2</sub> / 70% N<sub>2</sub> blend and 98% vacuum (lower oxygen), Haile et al. found better  $L^*$  scores and nitrosomyoglobin values (estimated by using the ratio of reflectance at specific wavelengths) for the lower oxygen MAP package. (Haile et al., 2013)  $L^*$  values from this study are reviewed in the next section.

#### 4.2.4b - $L^*$ analysis Test 2a

A summary of  $L^*$  values is found in Table 4.37. On a day by day comparison, the control (less than 0.5% O<sub>2</sub>) sample has the higher  $L^*$  value, which translates to lighter / faded appearance.

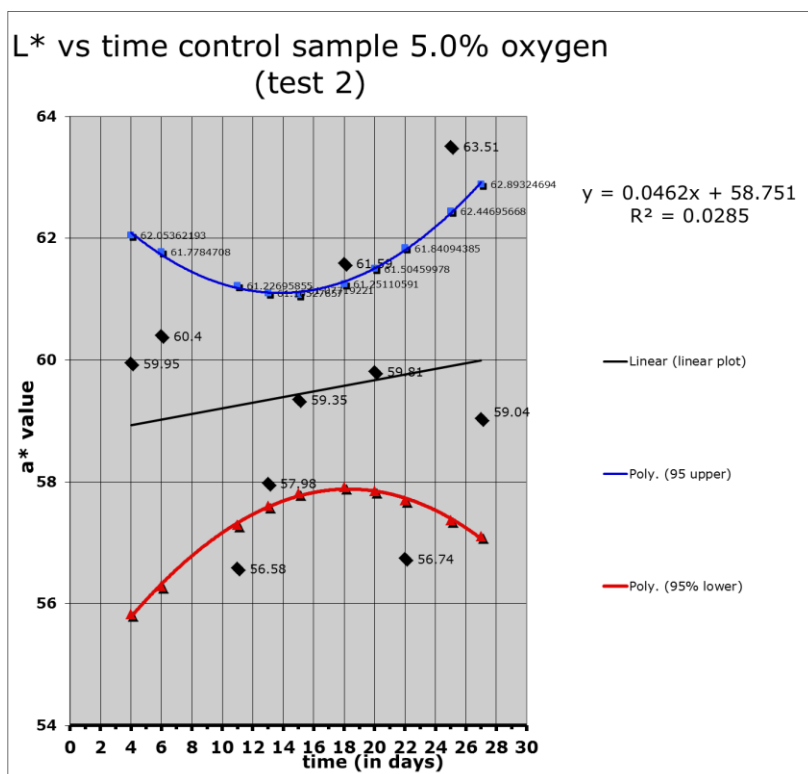
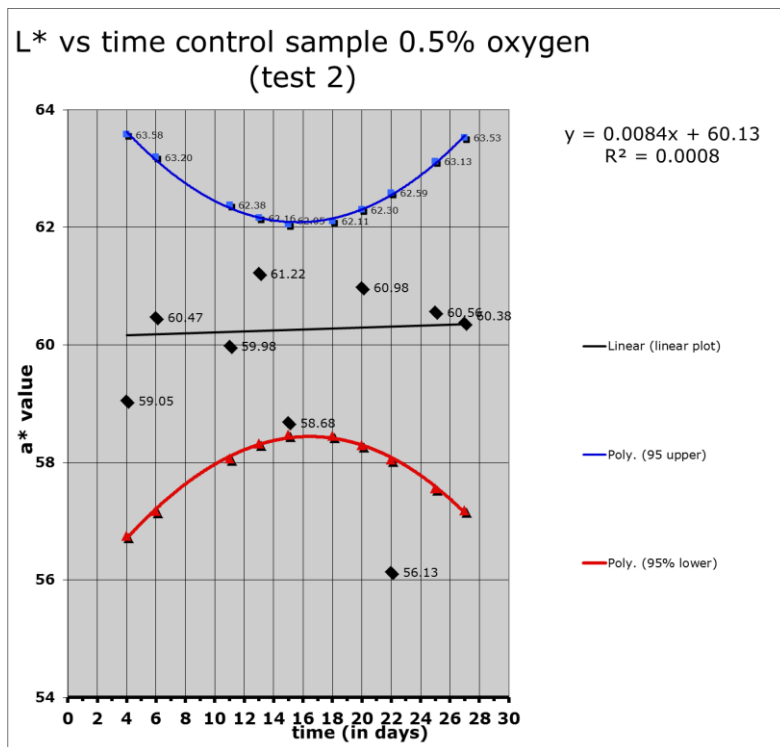
**Table 4.37** Summary of  $L^*$  values in Test 2a (0.5% vs 5.0% O<sub>2</sub>)

Day	Date	Control $L^*$	5.0% O <sub>2</sub> $L^*$	$\Delta L^*$ (control - 5% O <sub>2</sub> )	interpretation
Day 1	3/9/2012	56.83	59.55	-3	5% O <sub>2</sub> sample is lighter / faded
Day 4	3/12/2012	61.22	58.40	3	control is lighter / faded
Day 6	3/14/2012	59.05	59.95	-1	5% O <sub>2</sub> sample is lighter / faded
Day 8	3/16/2012	60.47	60.40	0	control is lighter / faded
Day 11	3/19/2012	59.98	56.58	3	control is lighter / faded
Day 13	3/21/2012	61.22	57.98	3	control is lighter / faded
Day 15	3/23/2012	58.68	59.35	-1	5% O <sub>2</sub> sample is lighter / faded
Day 18	3/26/2012	65.20	61.59	4	control is lighter / faded
Day 20	3/28/2012	60.98	59.81	1	control is lighter / faded
Day 22	3/30/2012	56.13	56.74	-1	5% O <sub>2</sub> sample is lighter / faded
Day 25	4/2/2012	60.56	63.51	-3	5% O <sub>2</sub> sample is lighter / faded
Day 27	4/4/2012	60.38	59.04	1	control is lighter / faded
	min	56.13	56.58		
	max	65.20	63.51		
	range	9.06	6.93		

Entering the  $L^*$  values from Table 4.37 above into the kinetics data input sheet (Tables 4.38 – 4.39) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey or concentration of pigments (which is also an indication of moisture loss).



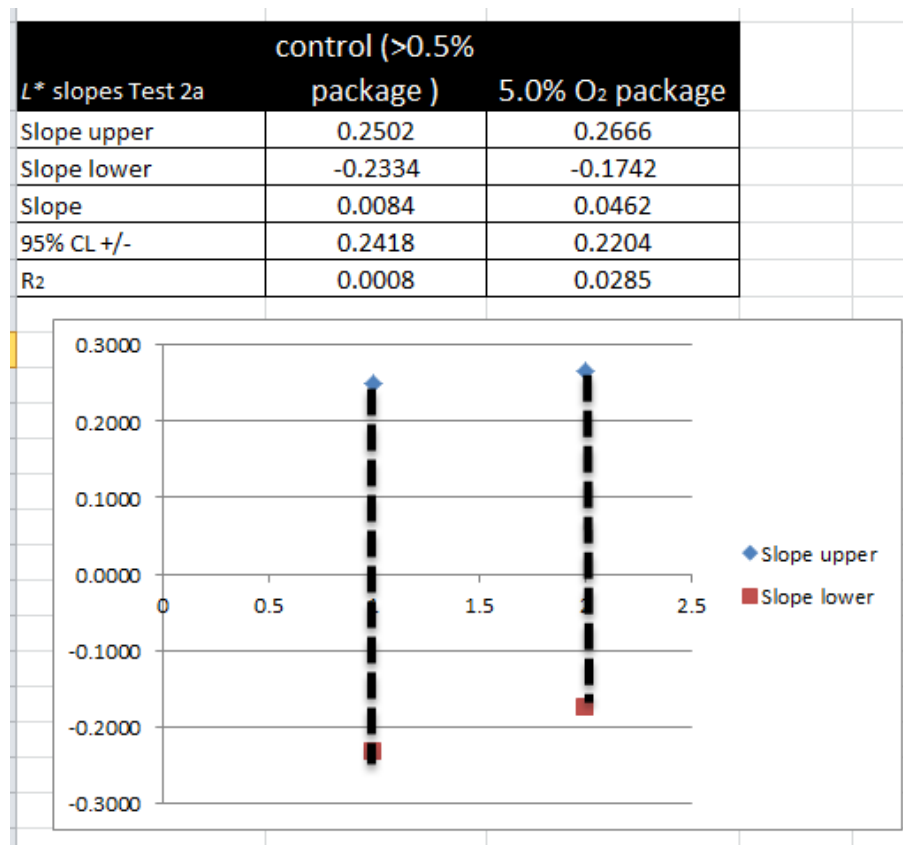




**Figure 4.35** Test 2 A: Control (0.5% O<sub>2</sub>) left, B: 5% oxygen; Zero order plot of  $a^*$  vs. time (30 days) with 95 % confidence limits calculation

Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges ( $+k$  for lightening over the shelf life,  $-k$  for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.40.

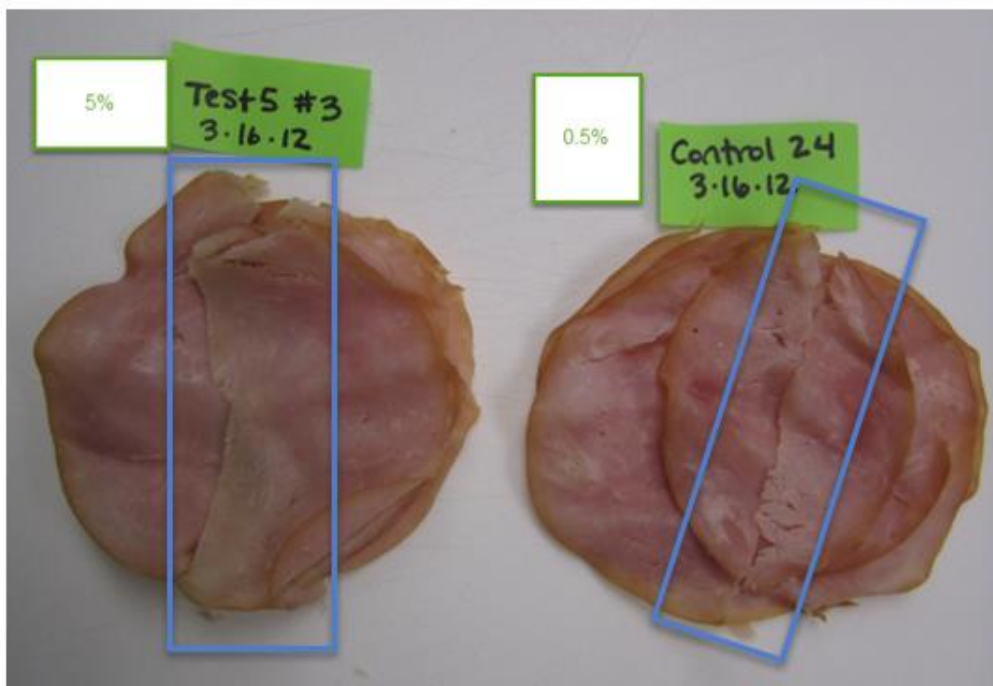
**Table 4.40**  $L^*$  parameter rate constant ( $k$ ) upper and lower for all applications in Test 2a all lanes as established by Labuza' Reaction kinetics shelf life model



In this sandwich study,  $L^*$  values over time were not significantly different for the 5.0% package compared to the control package, with both having very similar predicted rate constants, with a trend line slope increasing slightly over time (Figure 4.35).

#### 4.2.5a Visual appearance of ham Test 2a

The test samples packaged in the targeted 5.0% oxygen showed signs of discoloration at day 1 that continued throughout the study (Appendix B.2-B.8). The test samples targeting 0% oxygen showed signs of discoloration at day 11, but also contained high residual oxygen (0.65%) (Appendix B.1) The control samples that targeted 0.5% oxygen showed discoloration at day 13. These samples had low residual oxygen (0.0 and 0.1%), but were located near the light source (Appendix B.5). Comparing the day 8 samples of the control to the 5% residual O<sub>2</sub> headspace package, visual discoloration in the 5.0% package is readily seen by the naked eye (Figure 4.36) The (surface) depth of the discoloration is greater as predicted by the relationship between penetration beneath the surface and available oxygen partial pressure. The more oxygen there is in the headspace, the greater the depth of the penetration (Hunt et al., 2012).



**Figure 4.36** Day 8 comparison of control sample to 5.0% residual oxygen package in test 2.  $\Delta a^* = 4.66$  ( $a^*$  control –  $a^*$  5%). Actual residual oxygen in package control = 0.05%, 5% package = 2.57% The depth of discoloration is greater on the 2.57% package as depicted by the width of the grey color in the center of the slice where the surface interfaced with light exposure

#### 4.2.6a Cooler temperatures

AMSA defines non abuse temperatures as an average of 0-2° C. All coolers averaged non-abuse temperatures (Table 4.41). Temperature tracking for all coolers can be found in Appendix B.18.

**Table 4.41** The average cooler temperatures in Test 2. Note: control samples were stored in each cooler alongside the test condition

Cooler / product description	average temp C°	Min (C°)	Max (C°)
0% oxygen cooler	0.4	-3.5	3.0
5% oxygen cooler	0.1	-5.0	4.0

#### 4.2.7a Conclusions

This test supports the relationship of higher oxygen content in the headspace to the development of pigment discoloration in the meat. Based on the visual observations, the discoloration of ham in packages with > 2.5% O<sub>2</sub> starts earlier and is more complete than packages with >0.5% O<sub>2</sub>. The development of visual discoloration in the control samples was best seen at day 13 in the samples near the light source (Appendix B.5), while the 5% O<sub>2</sub> packages developed visual discoloration at day 1 (Appendix B.2). The limitation of the measurement method (Method 1 – Figure 3.9) may explain why *a*\* value rate constant predictions were similar. It also demonstrates the need to find supplemental options to eliminate oxygen from the package as industrial equipment adjustments alone are not enough. Further evidence is presented regarding the importance of the proximity to the light bulb in the cooler. It also provided a directional link between visual discoloration and numerical data (*a*\* values). In both this test and testing by Anderson and Rasmussen, a  $\Delta a^* > 3$  was linked to visual discoloration as observed by the naked eye (Anderson and Rasmussen, 1992).

## **4.2b Test 2b – UV (Ultraviolet) barrier film blocking at 380 nm compared to non-UV blocking packaging**

### **4.2.1b Overview of Test 2b**

The goal of this test was to assess the impact of using UV barrier film for the development of cured ham discoloration. The UV film produced by Belmark provides an inhibitor system (proprietary) but employed in its final form as a film, it is FDA approved and will block all UV light up to 380 nanometers (Belmark). Frozen raw minced beef color has been demonstrated to benefit from UV protection (Anderson, Bertelsen, and Skibsted, 1988). Comparing product stored in a hydroxybenzophenone UV absorber with PE (Polyethylene) tube (blocking between 350 – 220 nm) to a PE tube, frozen minced beef exposed to fluorescent tubes (illumination of 520 lux) showed improved surface color over 34 days refrigerated shelf life as measured by a Hunterlab D-25 tristimulus colorimeter for Hunter *a*. An improvement in  $\Delta a$  of 1-2 points between treatments was demonstrated over time in the UV packed product (Anderson, Bertelsen, and Skibsted, 1988). The color pigment of raw beef is oxymyoglobin. The cured meat pigment nitrosylmyoglobin however has been found to not benefit from UV protection when visible light is also present (Møller, Bertelsen, and Skibsted, 2002). Using nitrosylmyoglobin and metmyoglobin in aqueous solution at oxygen contents of 0.1, 0.5, and 1.0% with an 20% CO<sub>2</sub> / 80% N<sub>2</sub> gas mixture, photooxidation of the pigment was found to depend linearly on the amount of oxygen in both the visible (436 nm) and UV (366 nm) spectrum (Møller, Bertelsen, and Skibsted, 2002).

### **4.2.2b Methods**

The same methods used in test 2a were utilized. Both test 2a and 2b were run at the same time. A third cooler C was set up for the UV barrier package and control package (Table 4.42).

**Table 4.42** Test 2b cooler set up. A) Samples labeled C represent the control product (0.5% oxygen in the headspace). Cooler C samples shaded in pink represent the UV packaged samples (0.5% oxygen) B) Samples pulled on corresponding days

Cooler C								
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47
		C48	C49	C50	C51	C52	C53	C54
		C55	C56	C57	C58	C59	C60 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

Day	Date	Evaluation	Sandwiches for Color	Control # pulled	UV # pulled
Day 0	3/8/2012		sandwiches not reviewed		
Day 1	3/9/2012	1	1	27, 7, 47	6
Day 2	3/10/2012		sandwiches not reviewed		
Day 3	3/11/2012		sandwiches not reviewed		
Day 4	3/12/2012	2	2	26, 6, 46	5
Day 5	3/13/2012	3	sandwiches not reviewed		
Day 6	3/14/2012	4	3	25, 5, 45	4
Day 7	3/15/2012	5	sandwiches not reviewed		
Day 8	3/16/2012	6	4	24, 4, 44	3
Day 9	3/17/2012		sandwiches not reviewed		
Day 10	3/18/2012		sandwiches not reviewed		
Day 11	3/19/2012	7	5	23, 3, 43	14
Day 12	3/20/2012	8	sandwiches not reviewed		
Day 13	3/21/2012	9	6	34, 14, 54	13
Day 14	3/22/2012	10	sandwiches not reviewed		
Day 15	3/23/2012	11	7	33, 13, 53	12
Day 16	3/24/2012		sandwiches not reviewed		
Day 17	3/25/2012		sandwiches not reviewed		
Day 18	3/26/2012	12	8	32, 12, 52	11
Day 19	3/27/2012	13	sandwiches not reviewed		
Day 20	3/28/2012	14	9	31, 11, 51	10
Day 21	3/29/2012	15	sandwiches not reviewed		
Day 22	3/30/2012	16	10	30, 10, 50	20
Day 23	3/31/2012		sandwiches not reviewed		
Day 24	4/1/2012		sandwiches not reviewed		
Day 25	4/2/2012	17	11	39, 19, 59	19
Day 26	4/3/2012	18	sandwiches not reviewed		
Day 27	4/4/2012	19	12	38, 18, 58	18
Day 28	4/5/2012	20	sandwiches not reviewed		
Day 29	4/6/2012	21	13	37, 17, 57	17

#### 4.2.3b Oxygen content of the packages

The control and UV samples on average and at maximum values were within the range stated to be critical for oxygen (0.1 to 0.5%) by Møller et. al (Møller, Weber, and Bertelsen, 1999) (See Table 4.43). Raw data for CO<sub>2</sub> and O<sub>2</sub> is found in Appendix B.1.

**Table 4.43** headspace oxygen average, min, and max for Test 2b with UV barrier

Item	Average % O <sub>2</sub>	Min % O <sub>2</sub>	Max % O <sub>2</sub>
Test UV film	0.07	0	0.32
Control package	0.02	0	0.05

All samples at day 1 of refrigeration were between 0.01 – 0.16% O<sub>2</sub> (Appendix B.1). The Oxygen Transmission Rate (OTR) of all packaging was low (<.5 cm<sup>2</sup>/m<sup>2</sup>/atm/24h), and the product to head space ratio was 1 to 1. Given these three critical parameters were met and discoloration still occurred, the data would suggest that the critical level of oxygen in the package to prevent discoloration may be even lower yet for ham sandwiches, (all 20 sandwiches in the study developed visual discoloration in the 30 day refrigerated shelf life.

#### 4.2.4b *L\** and *a\** analysis Test 2b

The *a\** value performance of the UV barrier sample was similar to the control with the exceptions of day 4, 6, and 22 (Table 4.44). The location in the cooler for day 22 was near the light bulb, but days 4 and 5 were not. The oxygen headspace content of the UV barrier package was 0.0% at day 22.

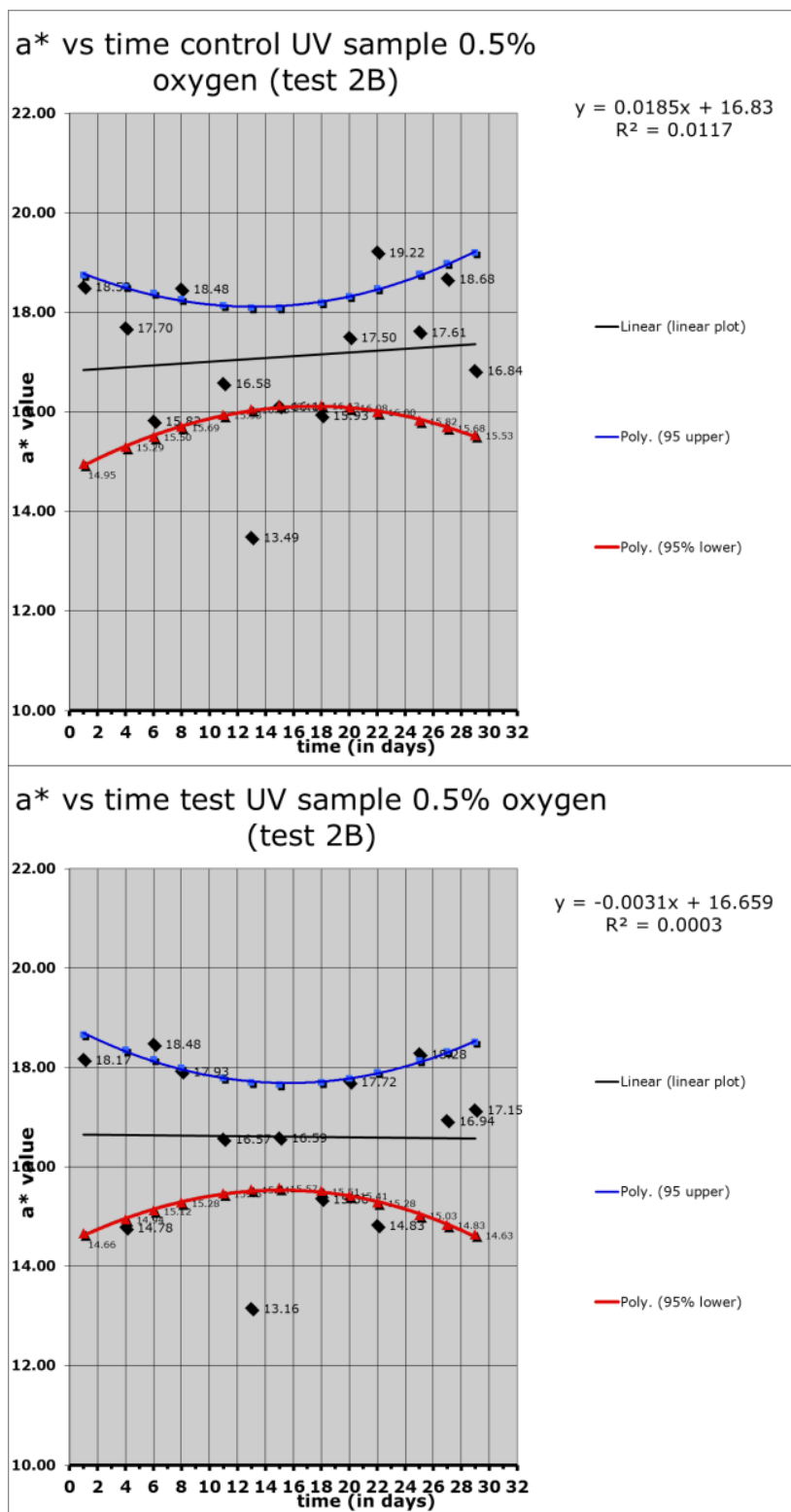
**Table 4.44** Test 2b - *a\** & *L\** value over time for control and UV package

day	Measurement	<i>L*</i>	<i>a*</i>	Measurement	<i>L*</i>	<i>a*</i>
1	Control package 47	60.08	18.52	Test UV #6	59.90	18.17
4	Control package 46	59.85	17.70	Test UV #5	63.10	14.78
6	Control package 45	61.19	15.82	Test UV #4	60.63	18.48
8	Control package 44	59.95	18.48	Test UV #3	59.63	17.93
11	Control package 43	62.20	16.58	Test UV #14	58.07	16.57
13	Control package 54	62.79	13.49	Test UV #13	63.47	13.16
15	Control package 53	61.00	16.11	Test UV #12	56.65	16.59
18	Control package 52	62.92	15.93	Test UV #11	60.54	15.36
20	Control package 51	61.28	17.50	Test UV #10	58.68	17.72
22	Control package 50	55.30	19.22	Test UV #20	61.41	14.83
25	Control package 59	59.42	17.61	Test UV #19	58.32	18.28
27	Control package 58	58.26	18.68	Test UV #18	61.76	16.94

Entering the *a\** and *L\** values from Table 4.44 above into the kinetics data input sheet (Tables 4.45 – 4.48) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

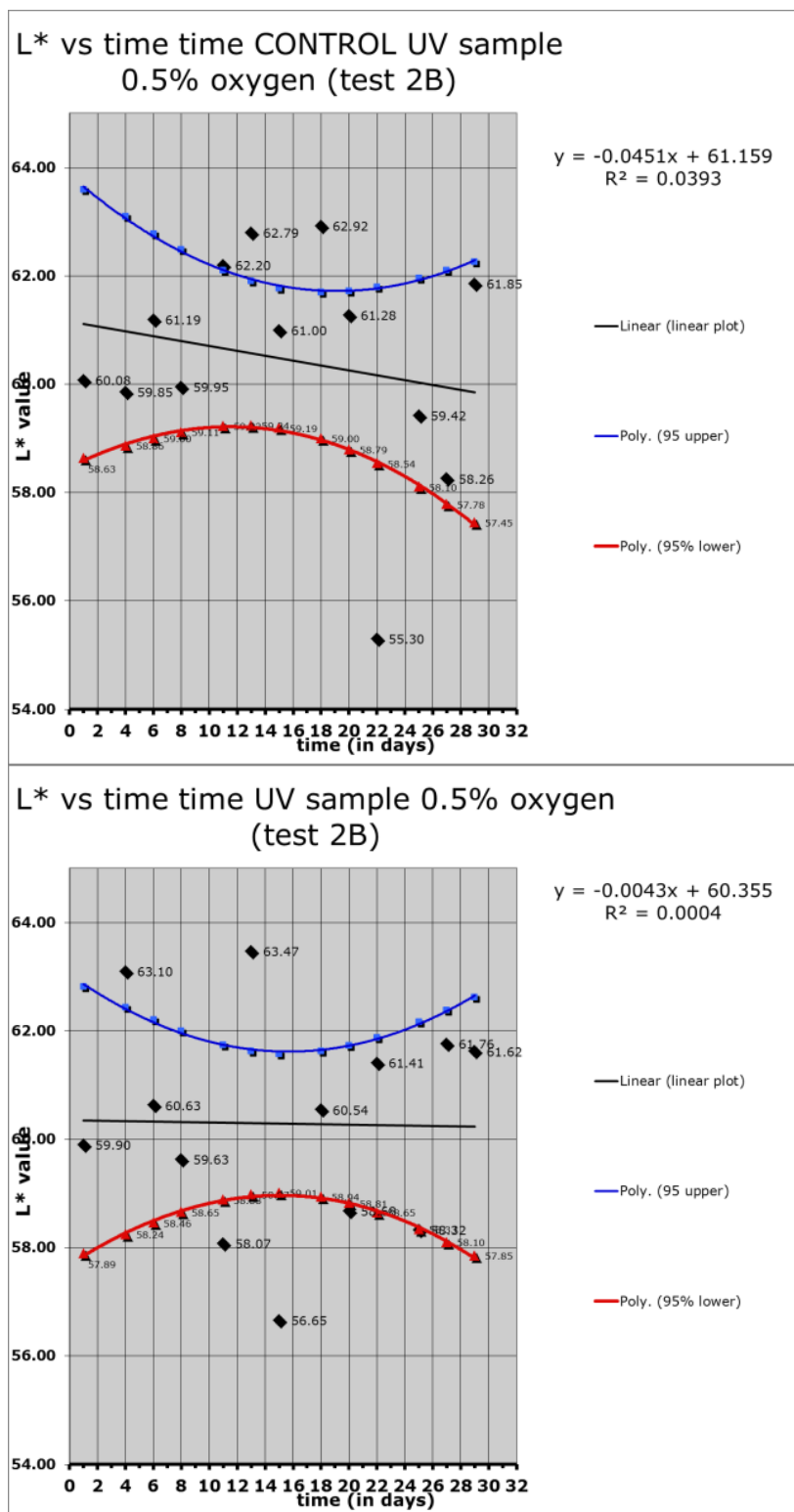






**Figure 4.37** Test 2 A: Control (0.5% O<sub>2</sub>) left, B: UV barrier (0.5% O<sub>2</sub>); Zero order plot of  $a^*$  vs. time (29 days) with 95 % confidence limits calculation

[illegible][illegible]



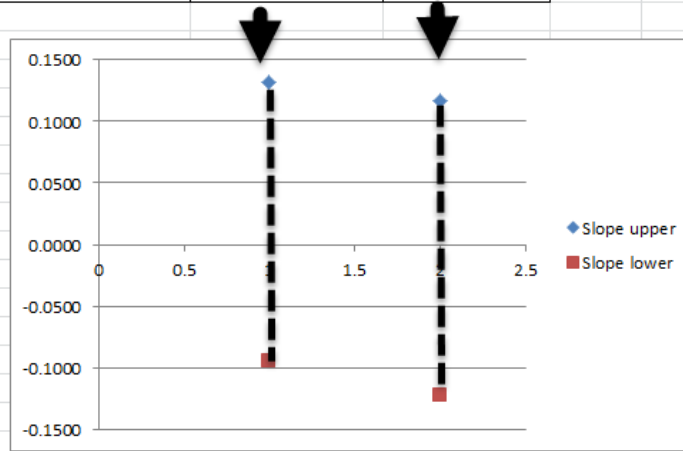
**Figure 4.38** Test 2 A: Control (0.5% O<sub>2</sub>) left, B: UV barrier; Zero order plot of L\* vs. time (29 days) with 95 % confidence limits calculation

**Table 4.49 A:** Test 2b  $a^*$  parameter rate constant (k) upper and lower for all applications all lanes as established by Labuza' Reaction kinetics shelf life model

B: Test 2b  $L^*$  parameter rate constant (k) upper and lower for all applications all lanes as established by Labuza' Reaction kinetics shelf life model

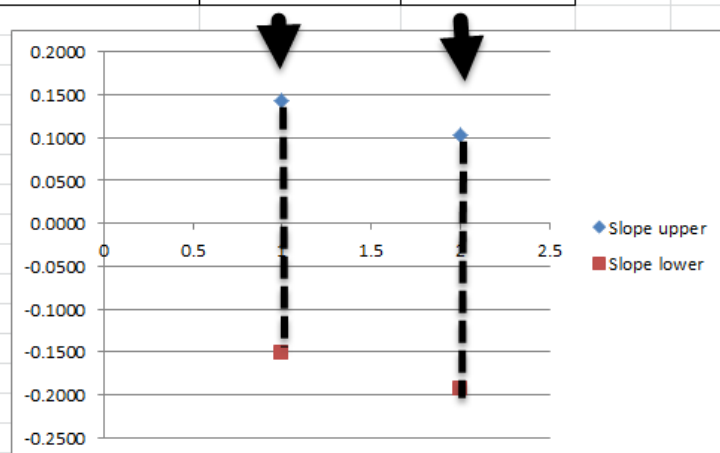
$a^*$ slopes Test 2b	control	UV film
Slope upper	0.1315	0.1163
Slope lower	-0.0945	-0.1225
Slope	0.0185	-0.0031
95% CL +/-	0.1130	0.1194
R2	0.0117	0.0003

A



$L^*$ slopes Test 2b	control	UV film
Slope upper	0.1427	0.1029
Slope lower	-0.1512	-0.1932
Slope	-0.0043	-0.0451
95% CL +/-	0.1469	0.1480
R2	0.0004	0.0393

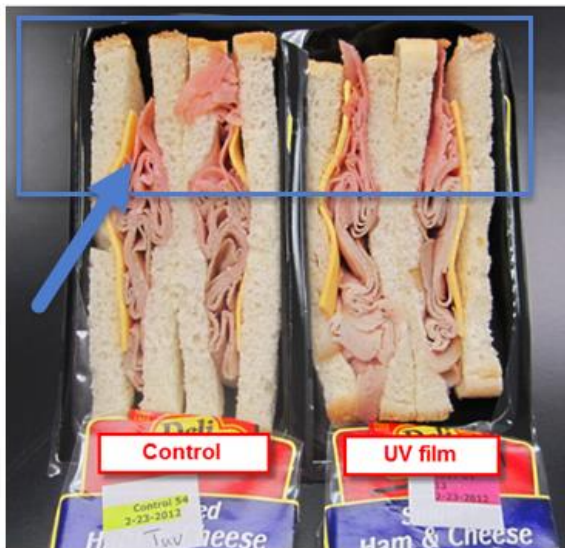
B



Statistically there was not a difference in  $a^*$  value or  $L^*$  value over time at the 95% confidence level (Table 4.49). The fit of the data was not good as measure by  $R^2$  on either  $a^*$  or  $L^*$  parameters (Figure 4.37 and 4.38). Both the UV and control samples scored low for  $a^*$  value at day 13. These samples were nearest the light source (Appendix B.13).

#### 4.2.5b Visual appearance of ham Test 2b

The visual appearance of product packaged in UV barrier film did not outperform the control sample. (Appendix B.10 – B.16) Both the control sample and the sample packaged in UV blocking film developed the washed out, greyish white discoloration indicative of photooxidation by days 11 and 13 (Appendix B.12 – B.13). This outcome supports Møller et al. findings that photo-oxidation is impacted by both visible and UV light (suggesting that light above 380 nm was responsible for this discoloration in this example). (Møller, Bertelsen and Skibsted, 2002) The label coverage of a portion of the ham provides a within sample comparison that illustrates the degree of discoloration that occurs on both the UV test sample and control (Figure 4.39, area in blue box)



**Figure 4.39** Sandwiches at day 11 of refrigeration in Test 3, left side is a control; right side has UV film protection. The upper  $\frac{1}{4}$  of the sandwich represents where the label covered the meat

#### 4.2.6b Cooler temperatures

All coolers were at non-abuse temperatures (average = 0 - 2 C° as defined by the AMSA) (Hunt et al., 2012). The average, minimum and maximum temperatures are found Table 4.50. Temperature tracking charts are located in Appendix B.18.

**Table 4.50** The average cooler temperatures in Test 2b. Note: control samples were stored in each cooler alongside the test condition

Cooler / product description	average temp C°	Min (C°)	Max (C°)
UV cooler	0.3	-4.5	4.0

#### 4.2.7b Conclusions

The outcome of the testing would suggest that UV film alone is not enough to prevent discoloration. The day to day  $a^*$  and  $L^*$  value performance of the UV packaged sandwich was similar to the control over time, with nearly identical predicted rate constants at the 95% confidence level (Table 4.49).

Unlike frozen minced beef which had improved color stability with a UV barrier film blocking under 350 nm (which may have benefited from cold temperatures and the necessary activation temperature for thermal oxidation) (Anderson, Bertelsen and Skibsted, 1989), cured ham on sandwiches in refrigeration conditions resulted in visual discoloration by day 11 with UV barrier film (Appendix B.12) Beef may also behave differently because it has 2-3 times more myoglobin than ham (Johnston, Knight, and Ledward, 1992). Raw meat also does not benefit from the color stability that the curing process creates.

The outcome of this test is in agreement with Møller et al. who established that UV light and visible light are nearly equal in causing discoloration (Møller et al., 2002).

## **4.3 Test 3 Alternate ham formulations**

### **4.3.1 Overview Test 3**

The goal of this test was to evaluate alternative ham formulations for  $a^*$  and  $L^*$  color scores and development of visual discoloration over time. The study included adding ingredients with antioxidant capability (Rosemary, fruit extract (which contains both orange/brown pigment and antioxidant capability)) to the current John Morrell<sup>®</sup> cured ham formula used by Deli Express<sup>®</sup> and a ham formulation from an alternative manufacturer (Cargill<sup>®</sup>) with a different combination of muscles used (insides only). While the mechanism is not fully understood, antioxidants work by reacting with free radicals and prevent oxidation reactions from occurring (Richards, 2007). UV light can generate free radicals as well as provide energy to drive dissociation of nitric oxide from the nitrosylhemochrome complex leading to the formation of metmyoglobin. There are free radicals that are associated with positive formation of cured meat color (Pegg and Shahidi, 1997). This creates the potential for antioxidants to both help prevent meat discoloration and interfere with cured meat color development. Different muscles within the same animals vary in myoglobin content and result in variation in color development during curing (Ledward, 1992).

Raw sausage (made with pork foreleg (primarily outside muscles)) in MAP (Modified atmosphere Packaging) has been demonstrated to have improved meat color with the use of antioxidants in combination with changes of lighting conditions (fluorescent, vs. fluorescent with a UV filter). Addition of rosemary and ascorbic acid in the absence of black pepper to fresh pork sausage retarded discoloration in sausages under illumination with a UV filter but not under standard fluorescent lighting, extending the refrigerated shelf life from 8 to 16 days, where other combinations of light and antioxidants did not work (Martinez et al., 2006).

### **4.3.2 Methods and Materials Test 3**

Three Beverage Air coolers (Model # LV27 c) with fluorescent bulbs were used in this study. Each cooler contained a control, and one or two test variables. All sandwiches



were numbered to represent each test treatment location in the cooler (Table 4.51). The sample numbers marked “pic” (Table 4.51) were photographed daily in the package, and reviewed for  $L^*$ ,  $a^*$  and oxygen content on day 32. Each shelf was filled one sandwich deep at the front position.

**Table 4.51** Test 3 cooler set up. *Cooler A* numbers in yellow represent the current smoked ham formulation (full production batch) used in Deli Express® Ham & Cheese sandwiches, green represent John Morrell® current formulation produced in a smaller pilot plant batch, blue represents current formulation with fruit extract added (also made in a pilot plant). *Cooler B* numbers in yellow represent the current smoked ham formulation used in Deli Express Ham & Cheese sandwiches (full production batch), red represent current formulation with rosemary added (also made in a pilot plant). *Cooler C* numbers in yellow represent the current smoked ham formulation used in Deli Express Ham & Cheese sandwiches (full production batch), grey represents Cargill® ham. Numbers with the suffix “pic” indicate samples that were photographed in the package throughout the study

<b>Cooler A</b>							
Current ham	1	2	3	4	5	6	7
(full production)	8	9	10	11	12	13	14 pic
Current ham	1	2	3	4	5	6	7 pic
(pilot plant)	8	9	10	11	12	13	14
Fruit extract	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
<b>Cooler B</b>							
Current ham	15	16	17	18	19	20	21
(full production)	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Rosemary	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
<b>Cooler C</b>							
Current ham	36	37	38	39	40	41	42
(full production)	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Cargill ham	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21

The sandwiches were evaluated fourteen times throughout a thirty two day refrigerated shelf life for  $L^*$  and  $a^*$  color, residual oxygen in the headspace and visual appearance once taken out of the package, using methods outlined in Test 2. Table 4.52 provides the calendar and sample numbers evaluated.

**Table 4.52** Test 3 calendar with sample numbers (#) evaluated and corresponding day in shelf life.

Day (in shelf life)	Date of evaluation	Control (current ham formula full plant batch) # evaluated (in the order of cooler A,B, and C)	Control produced in pilot plant # evaluated	Current formula with fruit extract added # evaluated	Current formula with rosemary added # evaluated	Cargill # evaluated
Day 2	4/11/2012	7, 21, 42	6	6	6	6
Day 4	4/13/2012	6, 20, 41	5	5	5	5
Day 7	4/16/2012	5, 19, 40	4	4	4	4
Day 9	4/18/2012	4, 18, 39	3	3	3	3
Day 11	4/20/2012	3, 17, 38	2	2	14	14
Day 14	4/23/2012	2, 28, 49	1	1	13	13
Day 16	4/25/2012	1, 27, 48	14	14	12	12
Day 18	4/27/2012	13, 26, 47	13	13	1	1
Day 21	4/30/2012	12, 25, 46	12	12	10	10
Day 23	5/2/2012	11, 24, 45	11	11	21	21
Day 25	5/4/2012	10, 34, 55	10	10	20	20
Day 28	5/7/2012	9, 33, 54	9	9	19	19
Day 30	5/9/2012	8, 32, 53	8	8	18	18
Day 32	5/11/2012	14, 31, 52	7	7	17	17

The process of establishing ham formulations to test was accomplished by collaborating with the John Morrell<sup>®</sup> Research and Development team. Initially six formulations were proposed. The initial choice of formulations was based on selecting natural antioxidants that could qualify as “flavorings” in the ingredient statement. A pilot plant batch of the current ham formula was produced as a control to minimize production variation for comparing to the test formulas. A full scale production sample was pulled from Deli Express<sup>®</sup> inventory to compare the consistency to the pilot plant sample. Both the John Morrell<sup>®</sup> pilot plant and full scale production facilities are located in Sioux Falls, SD. Initially considered formulations were:

- Current formula made in the pilot plant (to minimize any pilot plant batch variability)
- 0.014% rosemary extract (Tradename Oxy'less<sup>®</sup> CS (Appendix C.16)) + process change – this formula was not used in this test.
- 0.200% fruit extract Acerola fruit 17 (Appendix C.17)

- 0.015% rosemary extract (Tradename Oxy'less<sup>®</sup> CS (Appendix C.16)) – this formula was not used in this test.
- 0.030% rosemary extract (Tradename Oxy'less<sup>®</sup> CS (Appendix C.16)) – this formula was not used in this test.
- 0.030% rosemary extract (Tradename Oxy'less<sup>®</sup> CS (Appendix C.16)) + process change

Because of the limited number of spaces available for refrigerated storage, the pilot plant version of the current formula (representing the true control), fruit extract added formula and 0.030% rosemary extract + process change (order of adding the ingredients and maximum amount of rosemary) formula were selected from the above to test. All products were produced in 50 lb. batches on 2/08/12 at the John Morrell<sup>®</sup> pilot plant in Sioux Falls, SD using a high barrier casing (OTR 20cc @73°F (1 atm., m<sup>2</sup>, 24 hours at 0 % relative humidity)). The control (full scale production) ham casing in the Deli Express current formula has an OTR (Oxygen Transmission Rate) = 3 cc/m<sup>2</sup>/24hr/atm.

All sandwiches were assembled and packaged using a Multivac (R530) on the production floor at E.A. Sween Company on 3/20/12. The targeted residual oxygen level immediately following packaging was <0.5 % for all treatments. All sandwiches were assembled in the wedge format as the consumer would purchase (meat is bunched, placed between two slices of bread with cheese and cut on the bias to expose the middle cross section of the sandwich). The age of the ham log prior to slicing for the John Morrell<sup>®</sup> pilot plant products (which included the pilot plant control, the fruit extract added and rosemary added versions) was 40 days old. The production scale control ham log was 28 days old at the time of slicing. The age of the Cargill ham log was not established at the time of slicing. All hams were stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH). The length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The length of time of frozen dark storage for the sandwich from assembly to the start of the refrigerated shelf life test was 21 days.

The ingredient statements, muscles used and plant locations for each of the hams are listed in Table 4.53.

**Table 4.53** Cured ham details in Test 3

Sample	Manufacturer	Produced in:	muscles used	Ingredient deck
Control ham pulled from Deli Express production	John Morrell®	Full scale production, Sioux Falls, SD	Inside muscles with ground shank and kernal	A Portion of Ground Ham Shank and Ham Added [Cured with Water, Salt, Contains 2% or less of Modified Food Starch, Corn Syrup, Dextrose, Potassium Lactate, Sodium Lactate, Sugar, Sodium Phosphates, Sodium Diacetate, Sodium Erythorbate, Sodium Nitrite].
current formula made in the pilot lab	John Morrell®	Pilot plant, Sioux Falls, SD	Inside muscles with ground shank and kernal	A Portion of Ground Ham Shank and Ham Added [Cured with Water, Salt, Contains 2% or less of Modified Food Starch, Corn Syrup, Dextrose, Potassium Lactate, Sodium Lactate, Sugar, Sodium Phosphates, Sodium Diacetate, Sodium Erythorbate, Sodium Nitrite].
current formula with 0.200% fruit extract	John Morrell®	Pilot plant, Sioux Falls, SD	Inside muscles with ground shank and kernal	A Portion of Ground Ham Shank and Ham Added [Cured with Water, Salt, Contains 2% or less of Modified Food Starch, Corn Syrup, Dextrose, Potassium Lactate, Sodium Lactate, Sugar, Sodium Phosphates, Sodium Diacetate, Sodium Erythorbate, Sodium Nitrite]. <b>+ .2% fruit extract</b>
current formula with 0.030% rosemary extract + process change	John Morrell®	Pilot plant, Sioux Falls, SD	Inside muscles with ground shank and kernal	A Portion of Ground Ham Shank and Ham Added [Cured with Water, Salt, Contains 2% or less of Modified Food Starch, Corn Syrup, Dextrose, Potassium Lactate, Sodium Lactate, Sugar, Sodium Phosphates, Sodium Diacetate, Sodium Erythorbate, Sodium Nitrite]. <b>+ .03% Rosemary</b>
Cargill® ham (alternate supplier / formula)	Cargill®	Full scale production. Nebraska City, NE	Denuded ham inside muscles with ground shank	Cured with: Water, Dextrose, Salt, Contains less than 2%: Sodium Lactate, Sodium Phosphate, Sodium Diacetate, Sodium Erythorbate, Sodium Nitrite.

The cheese, bread, and packaging utilized are as described in Chapter 3 Methods and materials (3.1, 3.3 and 3.4B). All materials used were from the same production lot codes for consistency and to minimize batch variation.

### 4.3.3 Oxygen (O<sub>2</sub>) percentage per package Test 3

With the exception of packages established as “leakers” (packages with higher oxygen percentages over time due to seal failure (highlighted in red and yellow in Table 4.54), the sandwiches in this study ranged from 0 to 0.534% residual oxygen throughout the 32 day refrigerated shelf life (Table 4.54). There is substantial variability per package for oxygen content. The causes of variability are as described in Test 2.

**Table 4.54 A:** Oxygen percentages per package for all treatments in Test 3. Yellow color code boxes indicate potential leaker packages. Red indicates verified leaker packages

day	Cooler A Current formula (full scale production batch) O <sub>2</sub> %	Cooler A current formula made in the pilot plant O <sub>2</sub> %	Cooler A Current formula with fruit extract added O <sub>2</sub> %	Cooler B Current formula (full scale production batch) O <sub>2</sub> %	Cooler B Current formula with Rosemary added O <sub>2</sub> %	Cooler C Current formula (full scale production batch) O <sub>2</sub> %	Cooler C Cargill formula O <sub>2</sub> %
2	0.137	0.048	0.150	0.141	0.044	0.083	5.100
4	0.167	0.073	0.112	0.155	0.096	0.084	0.144
7	0.534	0.119	0.298	0.114	0.142	0.168	0.101
9	0.242	0.083	0.130	0.081	0.128	0.165	0.071
11	0.089	0.275	0.269	0.145	0.100	0.150	0.121
14	0.395	0.160	0.098	0.055	0.120	0.081	0.061
16	0.197	0.103	0.093	0.069	0.085	0.065	0.073
18	0.197	0.070	0.027	0.106	0.116	0.070	0.081
21	0.265	0.028	0.007	0.031	0.092	0.092	0.749
23	0.108	0.036	0.002	0.024	0.000	0.022	0.000
25	0.093	0.021	0.001	0.000	0.006	0.070	0.002
28	20.600	0.098	0.034	0.007	0.002	0.051	0.001
30	0.125	0.075	0.001	0.036	0.036	0.013	0.004
32	0.000	0.000	0.000	0.001	0.000	0.000	0.000
<b>minimum</b>	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<b>maximum</b>	20.600	0.275	0.298	0.155	0.142	0.168	5.100
<b>range</b>	20.600	0.275	0.298	0.155	0.142	0.168	5.100

#### 4.3.4 $a^*$ values Test 3

In this evaluation, the control sample formula (current Deli Express<sup>®</sup> ham full scale production) was placed in each of the three coolers (A, B, and C). The  $a^*$  values for these controls are reported by the cooler they were placed in (Table 4.55).

All treatments had substantial variability in  $a^*$  values over time with the greatest range occurring in the production scale control sample from cooler C ( $a^*$  range (max.-min.) = 6.94) This was due to a sandwich in lane B at day 25 with  $a^* = 13.11$  (containing 0.07% residual oxygen). The least amount of  $a^*$  variation was from the production scale control sample from cooler A ( $a^*$  range = 3.57 after the leaker value was removed) (Table 4.55).

**Table 4.55**  $a^*$  values for all treatments over time in test 3. Values for leakers were removed (indicated by red highlight)

day	Cooler A	Cooler A	Cooler A	Cooler B	Cooler B	Cooler C	Cooler C
	Current formula (full scale production batch) $a^*$	current formula made in the pilot plant $a^*$	Current formula with fruit extract added $a^*$	Current formula (full scale production batch) $a^*$	Current formula with Rosemary added $a^*$	Current formula (full scale production batch) $a^*$	Cargill formula $a^*$
2	16.89	19.59	17.79	18.06	16.31	17.08	13.97
4	17.41	18.26	18.95	18.56	13.78	17.41	17.96
7	19.42	16.83	19.07	19.55	17.88	18.90	19.71
9	17.55	18.11	20.10	18.33	18.77	19.34	17.00
11	17.90	19.42	20.29	17.46	17.79	20.05	16.26
14	17.91	19.11	18.37	18.30	18.10	17.11	15.44
16	18.94	15.89	18.45	15.63	17.44	17.60	18.31
18	17.22	17.91	17.27	18.74	15.22	19.02	17.55
21	16.16	19.33	18.42	17.47	19.26	18.58	16.90
23	19.73	18.12	19.79	18.50	18.09	18.49	16.37
25	17.72	18.71	19.64	17.03	15.64	13.11	18.10
28	11.55	20.41	21.29	20.13	18.11	18.48	16.22
30	18.53	18.42	18.97	19.83	19.24	18.43	16.21
32	18.69	18.88	18.50	16.15	17.29	16.47	15.51
<i>max</i>	19.73	20.41	21.29	20.13	19.26	20.05	19.71
<i>min</i>	16.16	15.89	17.27	15.63	13.78	13.11	13.97
<i>range (max - min)</i>	3.57	4.52	4.02	4.50	5.48	6.94	5.75

The initial outcome was that the cooler A control had the largest range of  $a^*$  values (8.18 with the leaker  $a^*$  included). The leaker package had 20.6%  $O_2$  with  $a^* = 11.55$ . The cooler location of this sample (#9) is lane F (which is approximately 16" from the light source). This is evidence that significant discoloration can occur when away from the light source with no MAP (high oxygen). Removing this value from the data set improves the minimum score to 16.16 for the control in cooler A, and reduces the range to 3.57 (Table 4.55 above). Removing the leaker data point for the control (cooler A) results in a similar range of  $a^*$  values obtained in cooler B, but not cooler C (ranges in order were 3.57, 4.50, and 6.94) (Table 4.55 above).

Other leakers occurred in the Cargill® ham samples at day 2 resulting in  $a^*$  values of 13.97 (5.1%  $O_2$ ) and day 21 resulting in an  $a^*$  value of 16.90 (0.749%  $O_2$ ). The location of the day 2 Cargill sandwich was within one lane of the light source (Lane B), the day 21 sample was located in Lane E. The combination of high oxygen and close proximity to the light (lane B) resulted in a quick decrease in redness (lower  $a^*$ ) while the combination of higher than desired oxygen (>0.5%), but under 1%, and further away from the light did not decrease as sharply over a longer period of time. Excluding all leaker packages, the minimum  $a^*$  value for each treatment came at different days

throughout the shelf life and from sandwiches located within three lanes from the light source (lane being defined as the vertical row in the cooler) (Figure 4.40). Others have found Illumination is the most critical display parameter for a MAP product when the meat surface is exposed to approximately 1000 lux (Moeller, Weber, Bertelsen, 1999).

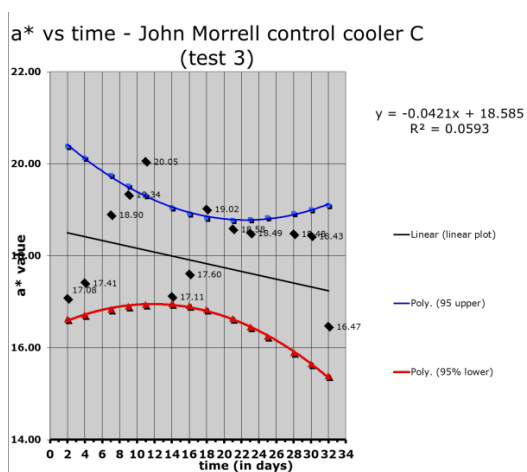
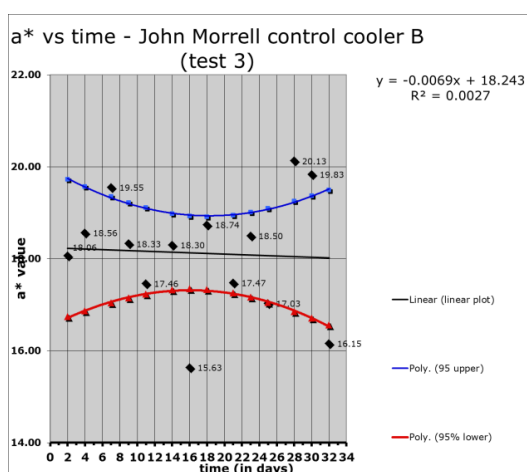
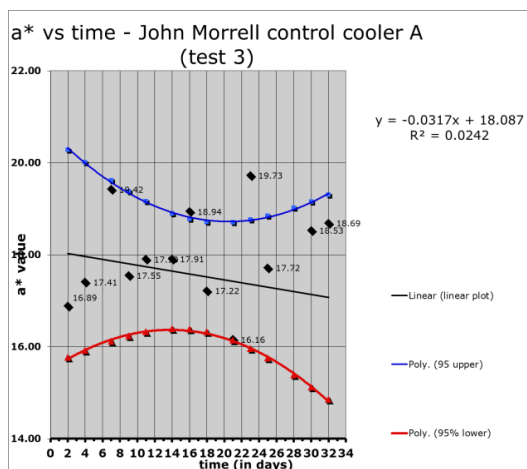
Cooler A	lanes	G	F	E	D	C	B	A
Current ham (full production)	1	2	3	4	5	6	7	pic
	8	9	10	11	12	13	14	pic
Current ham (pilot plant)	1	2	3	4	5	6	7	pic
	8	9	10	11	12	13	14	pic
Fruit extract	1	2	3	4	5	6	7	pic
	8	9	10	11	12	13	14	pic
Cooler B								
Current ham (full production)	15	16	17	18	19	20	21	
	22	23	24	25	26	27	28	
	29	30	31	32	33	34	35	pic
Rosemary	1	2	3	4	5	6	7	pic
	8	9	10	11	12	13	14	
	15	16	17	18	19	20	21	
Cooler C								
Current ham (full production)	36	37	38	39	40	41	42	
	43	44	45	46	47	48	49	
	50	51	52	53	54	55	56	pic
Cargill ham	1	2	3	4	5	6	7	pic
	8	9	10	11	12	13	14	
	15	16	17	18	19	20	21	

**Figure 4.40** Location of minimum  $a^*$  value for each test condition in Test 3 (as indicated by the black arrows). Lane A has 1 minimum  $a^*$  value, Lane B has 4 minimum  $a^*$  values, and Lane C has 2 minimum  $a^*$  values

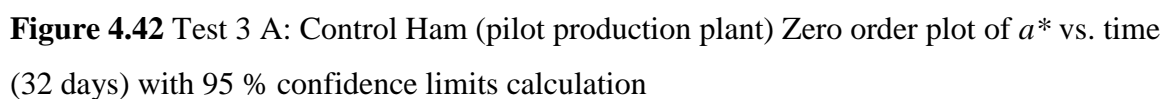
Entering the  $a^*$  values from Table 4.55 above into the kinetics data input sheet (Tables 4.56 – 4.60) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). This method also creates a line of best fit based on the actual point by point data that interprets the trend line for the attribute of interest (redness) over time (Figures 4.41-4.45). In this study, a negative (downward) slope indicates a decrease in redness ( $a^*$ ) or darkening of the product ( $L^*$ ), a positive (upward) slope indicates an increase in redness ( $a^*$ ) or lightening of the product ( $L^*$ ).

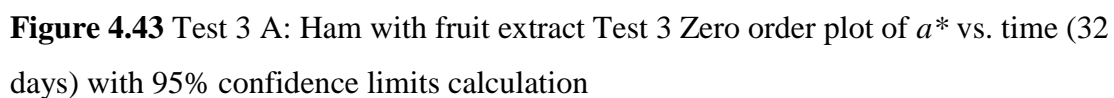






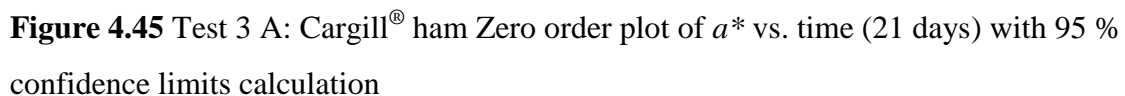
**Figure 4.41** Test 3 Zero order plot of  $a^*$  vs. time (32 days) with 95 % confidence limits calculation. A: Control Ham (production scale) test 3 from cooler A. B: Control Ham (production scale) from cooler B. C: Control Ham (production scale) from cooler C. All full scale production controls had a negative trend line indicating varying degrees of loss of redness over time.

[illegible]

[illegible]

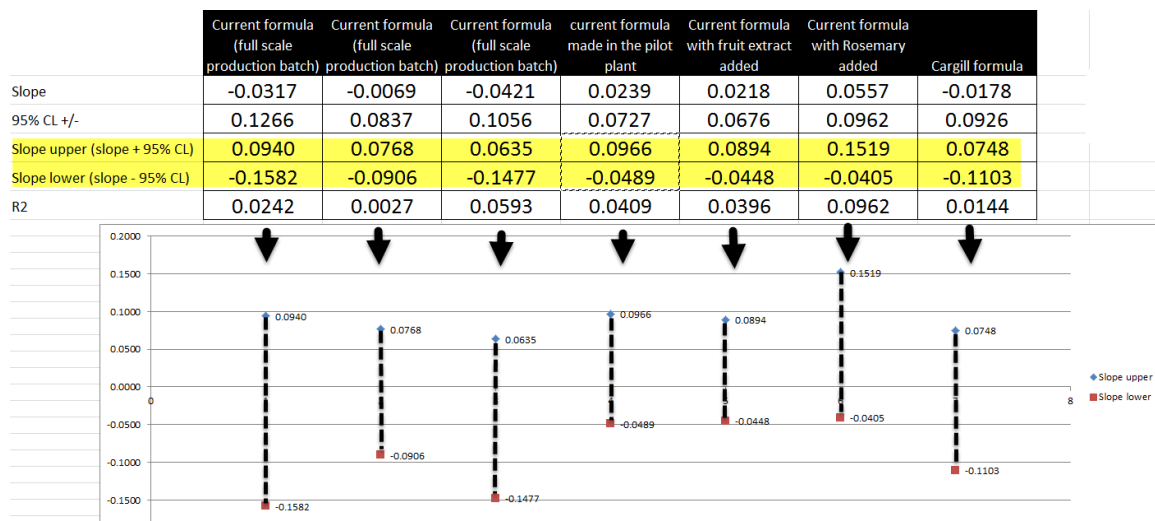


<b>1. Raw Data:</b>	Test 8 Cargill ham															
# data pairs Total=	14	This is automatically counted														
Y units	a*															
X units	days															
<b>2. Calculate Note after entering Y and X you need to pull down formulas in each column from top to last entry r(yi-yes)^2</b>																
	Y value	x= time	Y^2	Y plot value	Esi yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%LL	Delta	predicte average	
	13.97	2	195.07	13.97	17.09	4.00	13.97	17.09	9.76	229.31	27.93	4.00	18.74	15.44	3.30	17.09
	17.96	4	322.68	17.96	17.06	16.00	17.96	17.06	0.82	172.73	71.85	16.00	18.55	15.56	2.99	17.06
	19.71	7	388.62	19.71	17.00	49.00	19.71	17.00	7.35	102.88	137.99	49.00	18.28	15.72	2.56	17.00
	17.00	9	288.89	17.00	16.97	81.00	17.00	16.97	0.00	66.31	152.97	81.00	18.12	15.62	2.30	16.97
	16.26	11	264.39	16.26	16.93	121.00	16.26	16.93	0.45	37.73	178.86	121.00	17.97	15.89	2.08	16.93
	15.44	14	238.36	15.44	16.88	196.00	15.44	16.88	2.07	9.88	216.16	196.00	17.79	15.83	1.96	16.88
	18.31	16	335.13	18.31	16.84	256.00	18.31	16.84	2.14	1.31	292.81	256.00	17.72	15.97	1.75	16.84
	17.55	18	308.00	17.55	16.81	324.00	17.55	16.81	0.55	0.73	315.90	324.00	17.68	15.93	1.75	16.81
	16.90	21	285.50	16.90	16.75	441.00	16.90	16.75	0.02	14.88	354.83	441.00	17.69	15.81	1.88	16.75
	16.37	23	268.09	16.37	16.72	529.00	16.37	16.72	0.12	34.31	376.59	529.00	17.74	15.69	2.05	16.72
	18.10	25	327.73	18.10	16.68	625.00	18.10	16.68	2.02	61.73	452.58	625.00	17.82	15.55	2.27	16.68
	16.22	28	263.09	16.22	16.63	784.00	16.22	16.63	0.17	117.88	454.16	784.00	17.96	15.30	2.66	16.63
	16.21	30	262.87	16.21	16.59	900.00	16.21	16.59	0.14	165.31	486.40	900.00	18.07	15.12	2.95	16.59
	15.51	32	240.46	15.51	16.56	1024.00	15.51	16.56	1.11	220.73	496.21	1024.00	18.18	14.93	3.25	16.56
	Y value	x=time	Y^2	Y plot value	Esi yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%JL	y 95%LL	Delta	predicte average
slope=		-0.0178														
intercept=		17.1268														
rsq=		0.0144														
± 95% slope		0.0926														
k upper		0.0748														



Statistically, there was no difference in  $a^*$  color scores over a 32 day period for any of the treatments. Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments indicates that the treatment is not statistically different. A summary of the slope ranges (+k for increase in  $a^*$  value (redness) over the shelf life, - k for loss of redness or decreasing  $a^*$  value  $\pm$  95% CL) between treatments is provided in Table 4.61.

**Table 4.61**  $a^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in Test 3 as established by Labuza' Reaction kinetics shelf life model.



The three full scale production control samples (in coolers A, B, and C) have good repeatability in potential slopes. A similar consistency was noted in the three pilot plant treatments. All treatments have the potential of a negative slope (decreasing redness over time), but the full scale production controls and Cargill ham are more likely to be negative over time, while the pilot plant products have a greater potential to be positive. This suggests that there are other factors inherent to each batch that may be more important to the final color. Assuming the manufacturing process is consistent per batch (i.e. no difference in processing times or production steps); the age of the muscles going

into the batch may be an important factor. The average age of the hogs before slaughter used by John Morrell® is 6-7 months but varies batch to batch. If the age of the hogs used in the pilot plant batch were different than the full scale production batch, it may offer an explanation for the results (as myoglobin concentration is affected by age and diet of the animal).

The Cargill® ham performed in a range similar to the full scale production batch from John Morrell®.

The coefficient of determination ( $R^2$ ) indicates the fit of the data (or predictability) is poor for all treatments. In any case based on the low  $R^2$  for all the treatments ( $<0.12$ ) and the overlap of the slopes ( $k \pm 95\% \text{ CL}$ ), there is really no difference in the change of  $a^*$  value over time which is disappointing in part. This leads to a conclusion that any formulation change made involving muscle content or antioxidants considering methods to maintain color are not worth the effort and potential added cost. However, if the meat processor could improve the grinding and blending of the product to be more homogeneous, this might result in a starting product with less variability in the starting color of the ham, and would be worth reevaluating the products. The starting variability of the ham makes it more difficult to evaluate potential improvements for color. The current formulation is however based on consumer demand for a product that has organoleptic characteristics (taste, texture, appearance and smell) similar to whole muscle ham. Significant changes would likely affect sales.

The Cargill® ham samples contained only inside muscles, and no kernel (area by the cap sometimes referred to as the corner/kernel/tip that has a slightly deeper red color) which could have resulted in a lower starting  $a^*$  values throughout the study, however the predicted slope of the line over time was very similar to the control sample in cooler C. Although addition of the kernel to the products can potentially increase  $a^*$  value, it also introduces greater variability from slice to slice. There is no statistical evidence from this test that supports a product with all inside muscles will result in a more stable  $a^*$  color score over time. The sample with the fruit extract had the greatest potential for a positive slope over time. This may be attributed to the presence of pigments from the fruit which behave differently from red meat pigment for discoloration. Future tests may be to compare different ages of the hog at the time of slaughter; however manufacturer's

ability to guarantee consistency in age of muscles may not be business practical. Others have found the starting myoglobin concentration of the raw meat to be an important factor. Myoglobin concentration can vary with muscle type, diet and age of the animal (Moeller, Weber, Bertelsen, 1999)

#### **4.3.5 $L^*$ values Test 3**

$L^*$  values can change with formulation. Muscle shade (light and dark) is influenced by the age of the animal (older animals will be darker in color (smaller  $L^*$ ) because of increased myoglobin level with aging), sex, diet, and exercise. More frequently exercised muscles are darker in color, which creates muscle color variation in the same animal (Hunt et al., 2012; Ledward, 1992). For the full scale production control samples, there should be consistency in  $L^*$  score, but all of the pilot plant products (including the pilot plant control, fruit extract added and Rosemary formulas) and the Cargill product were all from separate production batches introducing the potential for differences based on manufacture process and materials used. In the case of the Cargill® ham, it is formulated with inside muscles only which are not used as much for support by the animal compared to the outside muscles, resulting in lighter initial color.

The  $L^*$  values for each cooler and days are shown in Table 4.62. Removing leaker package values, the greatest range in  $L^*$  value ( $\Delta L^* = 9.24$ ) was in the current formula with rosemary added. The smallest range in  $L^*$  value was with the Cargill® Ham ( $\Delta L^* = 3.22$ ) (Table 4.62)



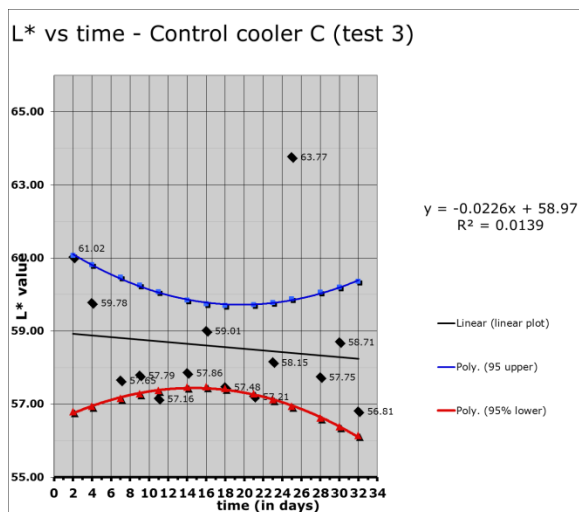
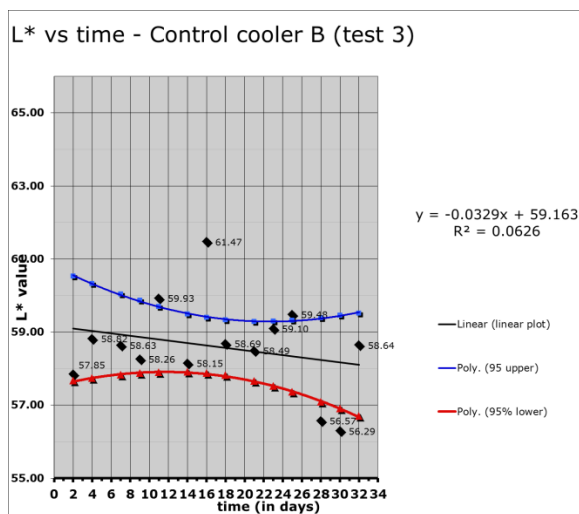
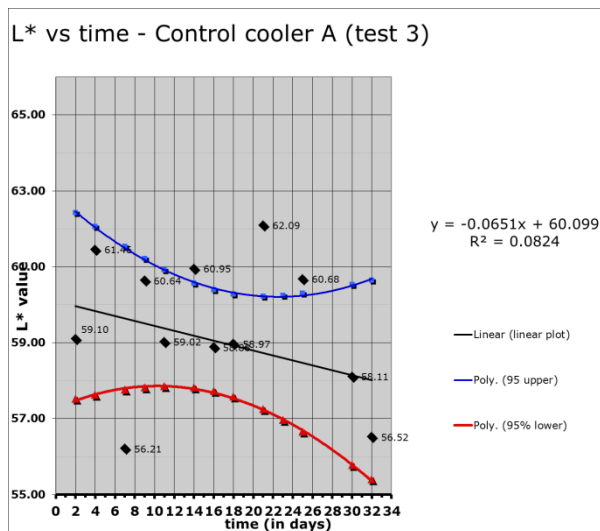
**Table 4.62** Test 3  $L^*$  values for all treatments (with minimum, maximum and range).

Red indicates where leaker values were removed

day	Cooler A	Cooler A	Cooler A	Cooler B	Cooler B	Cooler C	Cooler C
	Current formula (full scale production batch)	current formula made in the pilot plant	Current formula with fruit extract added	Current formula (full scale production batch)	Current formula with Rosemary added	Current formula (full scale production batch)	Cargill formula
	$L^*$	$L^*$	$L^*$	$L^*$	$L^*$	$L^*$	$L^*$
2	59.10	54.64	59.79	57.85	62.08	61.02	59.43
4	61.46	56.92	58.21	58.82	67.07	59.78	60.83
7	56.21	60.96	57.62	58.63	61.39	57.65	58.61
9	60.64	60.77	57.33	58.26	59.74	57.79	59.46
11	59.02	58.02	55.59	59.93	58.69	57.16	61.29
14	60.95	58.46	59.09	58.15	57.83	57.86	60.75
16	58.88	58.02	56.58	61.47	61.66	59.01	58.88
18	58.97	58.59	60.66	58.69	63.80	57.48	60.51
21	62.09	58.16	57.40	58.49	60.09	57.21	61.89
23	54.84	60.39	56.78	59.10	59.33	58.15	59.40
25	60.68	58.09	55.85	59.48	63.71	63.77	59.28
28	56.67	53.36	55.09	56.57	59.62	57.75	59.82
30	58.11	59.07	56.96	56.29	59.17	58.71	61.73
32	56.52	57.26	58.01	58.64	59.55	56.81	61.83
<i>minimum</i>	54.84	53.36	55.09	56.29	57.83	56.81	58.61
<i>maximum</i>	62.09	60.96	60.66	61.47	67.07	63.77	61.83
<i>range</i>	7.26	7.60	5.57	5.18	9.24	6.96	3.22

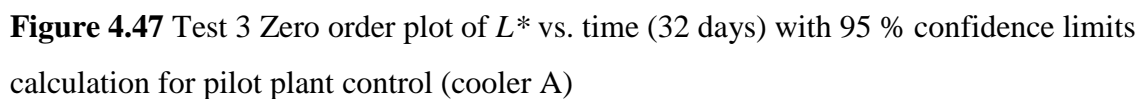
Applying the same method used for  $a^*$  values, establishes a predicted range of slopes for  $L^*$  value over the course of the shelf life at the 95% CL. Data input sheets for all treatments are listed in Table 4.63-4.67. Zero order plots of  $L^*$  over time are listed in Figures 4.46-4.50.





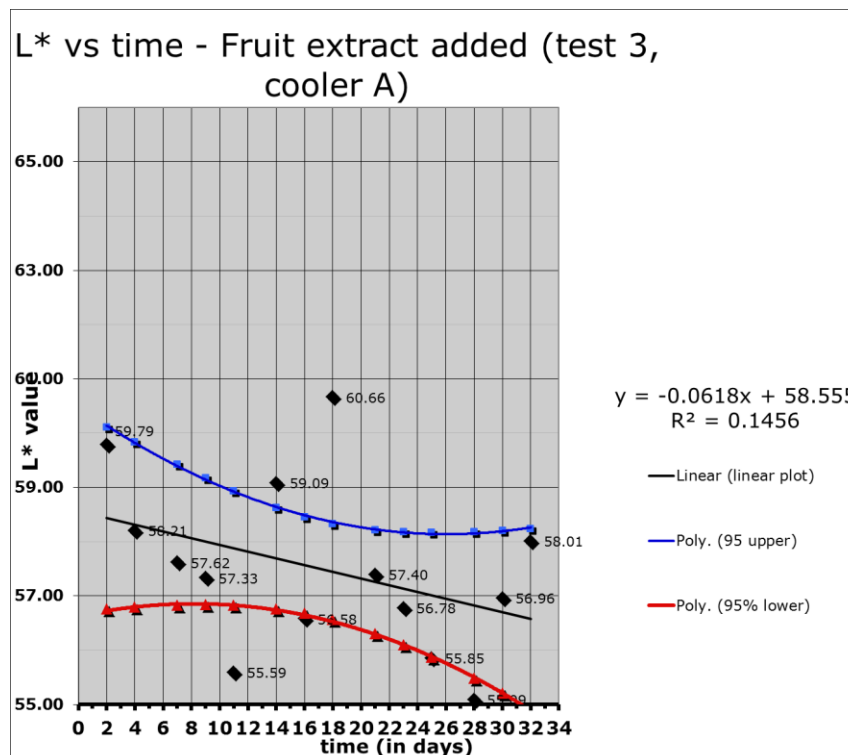
**Figure 4.46** Test 3 Zero order plot of  $L^*$  vs. time (32 days) with 95 % confidence limits calculation for A: Control cooler A, B: Control cooler B, C: Control cooler C

1. Raw Data:	Pilot plant contro																	
# data pairs	Total=	14	This is automatically counted															
Y units	L*																	
X units	days																	
STATISTICS																		
2. Calculati	Note after entering Y and X you need to pull down formulas in	each column from top to last entry row (y-yes)^2																
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte			
54.64	2	2985.53	54.64	58.31	4.00	54.64	58.31	13.44	229.31	109.28	4.00	60.74	55.88	4.86	58.31			
56.92	4	3239.89	56.92	58.27	16.00	56.92	58.27	1.83	172.73	227.68	16.00	60.48	56.07	4.41	58.27			
60.96	7	3716.12	60.96	58.22	49.00	60.96	58.22	7.50	102.88	426.72	49.00	60.11	56.34	3.77	58.22			
60.77	9	3692.59	60.77	58.19	81.00	60.77	58.19	6.65	66.31	546.90	81.00	59.88	56.49	3.39	58.19			
58.02	11	3365.93	58.02	58.15	121.00	58.02	58.15	0.02	37.73	638.18	121.00	59.68	56.62	3.06	58.15			
58.46	14	3417.96	58.46	58.10	196.00	58.46	58.10	0.13	9.88	818.49	196.00	59.45	56.75	2.70	58.10			
58.02	16	3365.93	58.02	58.07	256.00	58.02	58.07	0.00	1.31	928.27	256.00	59.36	56.78	2.58	58.07			
58.59	18	3432.40	58.59	58.03	324.00	58.59	58.03	0.31	0.73	1054.56	324.00	59.32	56.75	2.57	58.03			
58.16	21	3382.20	58.16	57.98	441.00	58.16	57.98	0.03	14.88	1221.29	441.00	59.37	56.60	2.77	57.98			
60.39	23	3646.55	60.39	57.95	529.00	60.39	57.95	5.94	34.31	1388.89	529.00	59.46	56.44	3.02	57.95			
58.09	25	3374.06	58.09	57.92	625.00	58.09	57.92	0.03	61.73	1452.17	625.00	59.59	56.25	3.34	57.92			
53.36	28	2846.93	53.36	57.86	784.00	53.36	57.86	20.32	117.88	1493.99	784.00	59.82	55.91	3.92	57.86			
59.07	30	3489.26	59.07	57.83	900.00	59.07	57.83	1.54	165.31	1772.10	900.00	59.85	55.66	4.34	57.83			
57.26	32	3278.33	57.26	57.80	1024.00	57.26	57.80	0.29	220.73	1832.21	1024.00	60.19	55.40	4.79	57.80			
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte			
slope=				-0.0170								Standard E	2.20					
Intercept=				58.3400								Sum (yi-yes	58.03					
rsq=				0.0061								n	14.00					
± 95% slope				0.1364								t 95% 2,n-2=	2.18					
k upper				0.1194								x average =	17.14					
k lower				-0.1534														
Equations												Sum (xi-xav	1235.71					
Y = 58.3400 - 0.0170 * time												(Sum x)^2	57600.00					

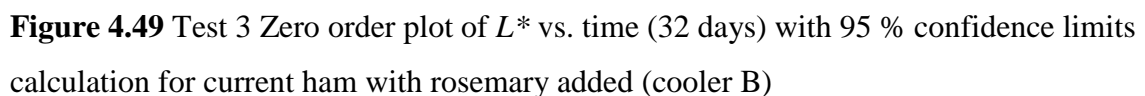


**Table 4.65** Test 3  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the current formula with fruit extract added

1. Raw Data:		Fruit extract															
# data pairs	Total=	14 This is automatically counted															
Y units	L*																
X units	days																
STATISTICS																	
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry																	
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average		
59.79	2	3574.45	59.79	58.43	4.00	59.79	58.43	1.84	229.31	119.57	4.00	60.11	56.75	3.36	58.43		
58.21	4	3388.02	58.21	58.31	16.00	58.21	58.31	0.01	172.73	232.83	16.00	59.83	56.79	3.04	58.31		
57.62	7	3319.68	57.62	58.12	49.00	57.62	58.12	0.26	102.88	403.32	49.00	59.42	56.82	2.60	58.12		
57.33	9	3286.73	57.33	58.00	81.00	57.33	58.00	0.45	66.31	515.97	81.00	59.17	56.83	2.34	58.00		
55.59	11	3090.62	55.59	57.88	121.00	55.59	57.88	5.21	37.73	611.53	121.00	58.93	56.82	2.11	57.88		
59.09	14	3491.23	59.09	57.69	196.00	59.09	57.69	1.95	9.88	827.21	196.00	58.62	56.76	1.87	57.69		
56.58	16	3200.92	56.58	57.57	256.00	56.58	57.57	0.98	1.31	905.23	256.00	58.46	56.68	1.78	57.57		
60.66	18	3680.04	60.66	57.44	324.00	60.66	57.44	10.37	0.73	1091.94	324.00	58.33	56.55	1.78	57.44		
57.40	21	3294.38	57.40	57.26	441.00	57.40	57.26	0.02	14.88	1205.33	441.00	58.21	56.30	1.91	57.26		
56.78	23	3223.97	56.78	57.13	529.00	56.78	57.13	0.13	34.31	1305.94	529.00	58.18	56.09	2.09	57.13		
55.85	25	3119.22	55.85	57.01	625.00	55.85	57.01	1.35	61.73	1396.25	625.00	58.16	55.86	2.31	57.01		
55.09	28	3034.91	55.09	56.83	784.00	55.09	56.83	3.01	117.88	1542.52	784.00	58.18	55.47	2.70	56.83		
56.96	30	3244.44	56.96	56.70	900.00	56.96	56.70	0.07	165.31	1708.80	900.00	58.20	55.20	3.00	56.70		
58.01	32	3365.16	58.01	56.58	1024.00	58.01	56.58	2.05	220.73	1856.32	1024.00	58.23	54.92	3.31	56.58		
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average		
slope=												-0.0618		Standard Error		1.52	
intercept=												58.5551		Sum (yi-yes)		27.68	
rsq=												0.1456		n		14.00	
± 95% slope												0.0942		t 95%,2,n-2=		2.18	
k upper												0.0324		x average =		17.14	
k lower												-0.1560					
Equations																	
Y = 58.5551												-0.0618		* time			
Sum (xi-xav)												1235.71					
(Sum x)^2												57600.00					
Sum(y^2)												46313.76					
sum y												804.95					
Sum (xi*yi)												13722.75					
sum x												240.00					
sum (X^2)												5350.00					



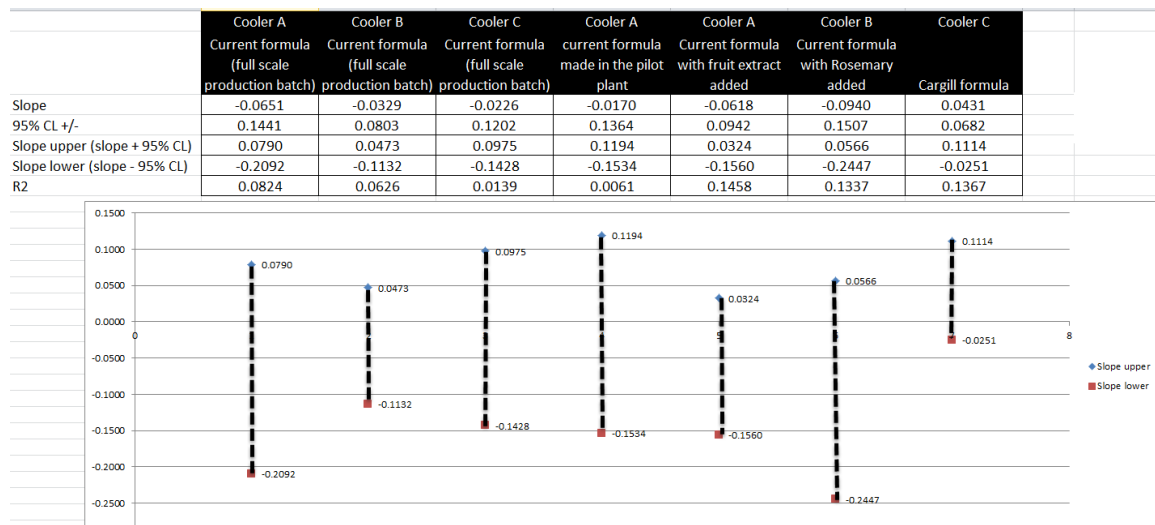
**Figure 4.48** Test 3 Zero order plot of  $L^*$  vs. time (32 days) with 95 % confidence limits calculation for current ham with fruit extract added (cooler A)

[illegible]



Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes in lightness and darkness as measured by  $L^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes ( $k \pm 95\%$  Confidence Levels (CL)) between treatments is not statistically different. A summary of the slope ( $+k$  for increase in  $L^*$  value (lightening) over the shelf life,  $-k$  for loss of redness or decreasing  $L^*$  value (darkening)  $\pm 95\%$  CL) overlap between treatments is provided in Table 4.68.

**Table 4.68**  $L^*$  Slope,  $\pm 95\%$  CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in Test 3 as established by Labuza' Reaction kinetics shelf life model.



$L^*$  values for all treatments have a greater potential for a negative slope (darker) over time with the exception of the Cargill<sup>®</sup> ham. Given the formulation difference, this fits given the lighter appearance of inside muscles compared to outside muscle. Statistically, there was no difference in  $L^*$  color scores over a 32 day period for any of the treatments. As hypothesized, the Cargill ham made with inside muscles trended towards being lighter in color over the shelf life. The control samples were not as consistent for  $L^*$  values between treatments as found in  $a^*$  values above, with the control sample from cooler C



performing similarly to the pilot plant control, and the rosemary added formula performing similarly to the control from cooler A over time (Table 4.68 above). Others have found better color retention in sliced ham based on  $L^*$  values, but not  $a^*$  (Chaiyapechara, Meng, and Hotchkiss, 1998).

#### 4.3.6 Visual appearance of ham Test 3

Visually, faded and grey discoloration was noted in all samples at different points throughout the study for the samples removed from the packaging. (Appendix C.1-C.13) Though the methodology of the sandwiches pulled didn't allow for a direct cooler location comparison (due to the sandwiches being held for daily pictures – See Table 4.51), in most instances, the samples nearest the light source developed visual fading or greying despite a low oxygen percentage in the headspace. For the samples with packaging photographed daily, no evident visual differences were noted from day to day (Appendix C.14).

#### 4.3.7 Cooler temperatures Test 3

All coolers averaged at non abuse temperatures over the course of the study (Table 4.69). The temperatures were consistent with the ranges established in Test 2.

**Table 4.69** Cooler temperatures for Test 3

Cooler	contained	Average (C°)	Min (C°)	Max (C°)
A	Control, pilot plant control, Ham with fruit extract	0.1	-5	4
B	Control, Ham with Rosemary	0.6	-3	3.5
C	Control, Cargill Ham	0.5	-4.5	4.5

#### 4.3.8 Conclusions Test 3

Of the three formulation changes considered (Current John Morrell® formula with rosemary, current John Morrell® formula with fruit extract, and Cargill® formula with inside muscles only), all three developed visual discoloration within the 32 day refrigerated shelf life, and none achieved a statistical difference in  $a^*$  or  $L^*$  over time compared to the current John Morrell® formula. The outcome was similar to a finding in fresh pork sausage. The addition of antioxidants like rosemary alone did not add protection from discoloration in fresh pork sausage under fluorescent lighting (Martinez et al., 2006). Only the combination of rosemary plus ascorbic acid without black pepper slowed discoloration under lighting with a UV filter (Martinez et al., 2006).

Antioxidants can act alone as a primary antioxidant or in synergy with other antioxidants (Hui, 2007). The mechanism of how they work is not fully understood, and there are many types of oxidation mechanisms including lipid oxidation, auto oxidation of the meat pigments, and photooxidation of meat pigments (Hui, 2007). The challenges of using antioxidants to help slow meat discoloration is 1) finding a primary antioxidant or synergistic combination effective for preventing the reaction of primary concern, 2) Establishing the right quantity to add (over time antioxidants will be depleted of hydrogen ions allowing oxidation to resume while adding too much catalyzes oxidation), 3) Creating the right conditions (pH is critical for controlling the rate of oxidation) (Labuza, 1971), and 4) Controlling what the antioxidant reacts with. While other combinations may exist, significant trial and error is required to come up with an effective combination, without a guarantee of success. With the desire to maintain the current bestselling formulation, this makes this option unappealing. There also is not enough evidence in the visual appearance of the test samples as compared to the control or the  $L^*a^*$  analysis to warrant further exploration.

The Cargill® ham sample demonstrates that muscle combinations used can result in visual differences and in  $L^*$  predicted ranges with a greater likelihood of lighter appearance; however variability still exists in the formula. With the kernel / tip removed, the Cargill® formulation visually was more consistent in appearance with fewer dark red spots, but still is not statistically better than the John Morrell meat formulation on the parameters of redness (as judged by  $a^*$ ) and light and dark contrast (as judged as  $L^*$ ).

## **4.4 Test 4 Ferrous based oxygen scavenging sachet**

### **4.4.1 Test 4 overview**

This test reviewed the use of an oxygen scavenger with Modified Atmosphere Packaging (MAP) to slow cured ham pigment from discoloration over time. The scavenger used was Multisorb D-30 (Appendix D.6). The type D design is patented, but the active ingredient is iron, and a salt and moisture source is included. The packet is activated by exposure to oxygen (which requires that the packets be vacuum packed in storage prior to adding to the sandwich). Requirements for optimal effectiveness are use of an adequate oxygen barrier film ( $<1$  cc of oxygen /  $100 \text{ in}^2/24$  hours), hermetic seals ( $3/8''$  wide), and free circulation around the product. This type of oxygen scavenger was designed for use with dry foods and contains its own source of moisture. It was selected for this application because the initiation time of the oxygen scavenging reaction is faster as it is not reliant on moisture from the food to initiate the reaction. In the moist scavenger application, both oxygen and water need to permeate the scavenger packaging to initiate the reaction. Because freezing will slow the scavenging reaction, the time available to quickly scavenge oxygen from the headspace is limited (from the time of manufacture until the product is frozen (Sandwiches achieve  $4.4 \text{ C}^\circ$  in approximately 4 hours, and reach  $-12 \text{ C}^\circ$  in approximately 24 hours)). The critical limitations that could prevent the scavenger from being effective in the sandwich package is the amount of time available before the sandwich freezes, and proper air flow around the scavenger (the sandwich rests on top of the packet in the package restricting the air flow around the scavenger). Oxygen scavengers have been proven effective in slowing meat discoloration in both raw and cured meats.

Buys found that raw pork when packaged in a 100%  $\text{CO}_2$  atmosphere with an oxygen scavenger (Ageless<sup>®</sup> R, Mitsubishi Gas Chemical Company incorporated, Tokyo Japan) achieved a color-life improvement of 5 days compared to a control without an  $\text{O}_2$  scavenger. This test used three methods to evaluate product including consumer acceptance panels, spectrophotometric reflectance (to calculate the metmyoglobin percentage) and color measurement with a Minolta chromameter. Color measurement data was not included in the results, and the conclusions of the study were based only on consumer acceptance panel results (Buys, 2004).

In a study of case ready raw beef steaks, meat acceptability failed within 7 days without an O<sub>2</sub> scavenger and permanent discoloration was observed. However with a Freshmax<sup>®</sup> scavenger included (Multisorb Technologies Inc., Buffalo NY), acceptable storage life was increased to as much as 21 days (Limbo et al., 2013).

In a study of sliced pasteurized ham packaged with an oxygen scavenger (Ageless<sup>®</sup> SS-50 and GM-50) and a low OTR film ( $2 \text{ cm}^3 (\text{m}^2 24 \text{ h atm}^{-3})$ ), discoloration of the ham was found to be completely eliminated in the first 24 hours of display compared to a control without a scavenger. Using a Hunterlab D-25 chromameter,  $a^*$  values of vacuum packed ham with an oxygen scavenger were shown to improve as much as  $\Delta a^* = 5$  points during the first 16 hours of storage when exposed to light (Anderson and Rasmussen, 1992).

In a study of sliced ham in combination with gas flush and vacuum using a low OTR film and an oxygen scavenger (Freshmax<sup>®</sup> Type B & M; Multisorb Technologies Inc., Buffalo NY), Chaiyapechara, Meng and Hotchkiss found lower psychotropic bacteria, yeast, and mold counts, and better color retention (in the form of Hunter L value as measured by Macbeth Coloreye chromameter) when comparing treatments with and without the O<sub>2</sub> scavenger (Chaiyapechara, Meng, and Hotchkiss, 1998).

In a study of sliced cooked ham in Polylactic Acid (PLA) trays, an oxygen scavenger combined with a CO<sub>2</sub> emitter increased shelf life up to 10 days at challenge temperatures of 6-8°C. Even better results were obtained when combined with MAP and a low O<sub>2</sub> level. Measured with a Minolta chromameter,  $a^*$  values of 11 to 15 were obtained with a 70% N<sub>2</sub> + 30% CO<sub>2</sub> MAP only, and 100% N<sub>2</sub> with O<sub>2</sub> scavenger and CO<sub>2</sub> emitter; whereas Non-MAP with CO<sub>2</sub> emitter and O<sub>2</sub> scavengers yielded  $a^*$  values = 8 to 9 (Cerioli et al., 2009).

#### **4.4.2 Methods and Materials**

Cooler A was filled with control packages (with a targeted O<sub>2</sub> level of <0.5%) and cooler B was filled with test packages that had the same O<sub>2</sub> level targeted but included a single oxygen scavenger packet per package. Six shelves were filled, one sandwich deep on the front edge of the shelf. Two Beverage Air coolers (Model # LV27 c) with fluorescent bulbs were used in this study. Two sandwiches were removed from each cooler on select

days (Table 4.70) and product was evaluated for  $L^*a^*b^*$  values and oxygen percentage in the headspace.

**Table 4.70** Test 4 Sample number evaluated and corresponding day in shelf life.

Day	Date	Evaluation	Control # pulled	Control # pulled	Test # pulled	Test # pulled
Day 3	6/18/2012		1	7	14	7 14
Day 5	6/20/2012		2	6	13	6 13
Day 7	6/22/2012		3	5	12	5 12
Day 10	6/25/2012		4	4	11	4 11
Day 12	6/27/2012		5	3	10	3 10
Day 14	6/29/2012		6	39	19	39 19
Day 17	7/2/2012		7	38	18	38 18
Day 24	7/9/2012		8	33	26	33 26
Day 26	7/11/2012		9	32	25	32 25
Day 28	7/13/2012		10	31	24	31 24
Day 31	7/16/2012		11	30	23	30 23

Visual appearance of the ham out of the package was reviewed during the first week of shelf life (results in Appendix D.1 – D.3). Sample number 20 was left in both coolers throughout the study, and photographed days 3 – 31 (Appendix D.4). Sealed packaged sandwiches in this study spent 7 days in frozen dark storage before being placed in refrigeration. All sandwich materials and packaging were taken from the same lot codes and produced at the same time to minimize differences between the control and test sample.

#### 4.4.3 Oxygen percentages per package Test 4

Three samples throughout the study were established as “leakers” (packages with incomplete seals resulting in oxygen levels in the headspace near atmospheric conditions). The data is included in Table 4.71 (leaker samples marked in red), but the color scores were discarded in the Lab\* analysis for these packages.

**Table 4.71** Oxygen percentages over time for control and scavenger samples in Test 4. The day in shelf life, sample number and corresponding oxygen percentage in the head space is recorded for all test and control samples

day	control #	O <sub>2</sub> %	control #	O <sub>2</sub> %	test #	O <sub>2</sub> %	Test #	O <sub>2</sub> %
3	7	0.080	14	0.149	7	21.700	7	0.020
5	6	0.094	13	0.048	6	0.000	6	0.000
7	6	0.044	12	19.700	5	0.000	5	0.000
10	4	0.103	11	0.131	4	0.000	4	0.000
12	3	0.053	10	0.069	3	0.000	3	0.000
14	19	0.122	39	0.072	19	0.000	19	0.064
17	18	0.076	38	0.063	18	0.000	18	0.000
24	26	0.071	33	0.044	26	0.000	26	15.300
26	25	0.059	32	0.062	25	0.000	25	0.000
28	24	0.083	31	0.025	24	0.000	24	0.000
31	23	0.046	30	0.067	23	0.000	23	0.000

For the remaining control samples, O<sub>2</sub> levels were within the range deemed critical to prevent discoloration (Table 4.71). Not all scavenger samples achieve 0.0% oxygen. (Table 4.71 samples marked in yellow) In addition to potential limited air flow and time available before a complete freeze is achieved, another possible explanation for this outcome is that the 30 cc removal capacity was not adequate. The estimation for the amount of O<sub>2</sub> needed to be removed (Table 3.4 in methods and materials) assumes 0% head space in the package with 25% void space (defined as air space between matter) in the sandwich and a residual 0.5% oxygen in the 768 cc package size (ps). With this combination of factors, an estimated 1 cc of oxygen needs to be removed from the package. If the void space (vs), head space (hs), and percent oxygen (O<sub>2</sub>%) in the headspace are greater, the cc of oxygen needed to be removed increases. A 30 cc capacity scavenger should be adequate, however estimating void space is challenging as bread is very porous, and headspace may increase as the freezing process can cause shrinkage. If the void space was as much as 50.0% with a 10.0% headspace, the percentage of oxygen needed to generate 30 cc of oxygen in a package would be 6.5% (cc of oxygen to be removed = ((vs\*ps) + (hs\*ps)) \* O<sub>2</sub>%). Because zero percent oxygen was not achieved in all packages (even at refrigerated temperatures), this suggests that air flow may not have been adequate around the scavenger in some sandwiches.

#### 4.4.4 $a^*$ values Test 4

Because of the sample pairings that were selected, there were not enough data points to statistically compare performance for product nearest the light source between applications (Table 4.72 – yellow highlighted indicates samples reviewed throughout the study).

**Table 4.72** Samples evaluated in test 4 (highlighted in yellow)

<b>Cooler A</b>								
Control	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
	Bottom shelf - 6	C35	C36	C37	C38	C39	C40 Pic	
<b>Cooler B</b>								
Test	Top - 1	1	2	3	4	5	6	7
		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
		21	22	23	24	25	26	27
		28	29	30	31	32	33	34
	Bottom shelf - 6	35	36	36	38	39	40 Pic	

The three leakers (sample numbers 7 ( $a^* = 11.05$ ), C12 ( $a^* = 12.18$ ), 26 ( $a^* = 17.13$ )) results in two of the three low  $a^*$  value scores (Table 4.73). With the leaker  $a^*$  value scores removed, the range of  $a^*$  scores for the control and scavenger samples are similar with the top shelf controls having a  $\Delta a^* = 2.12$  and the scavenger  $\Delta a^* = 2.14$  for the top shelves, and the control bottom shelves at  $\Delta a^* = 3.34$  and the scavenger bottom shelves at  $\Delta a^* = 2.91$ .  $L^*a^*b^*$  raw data is located in Appendix D.7.

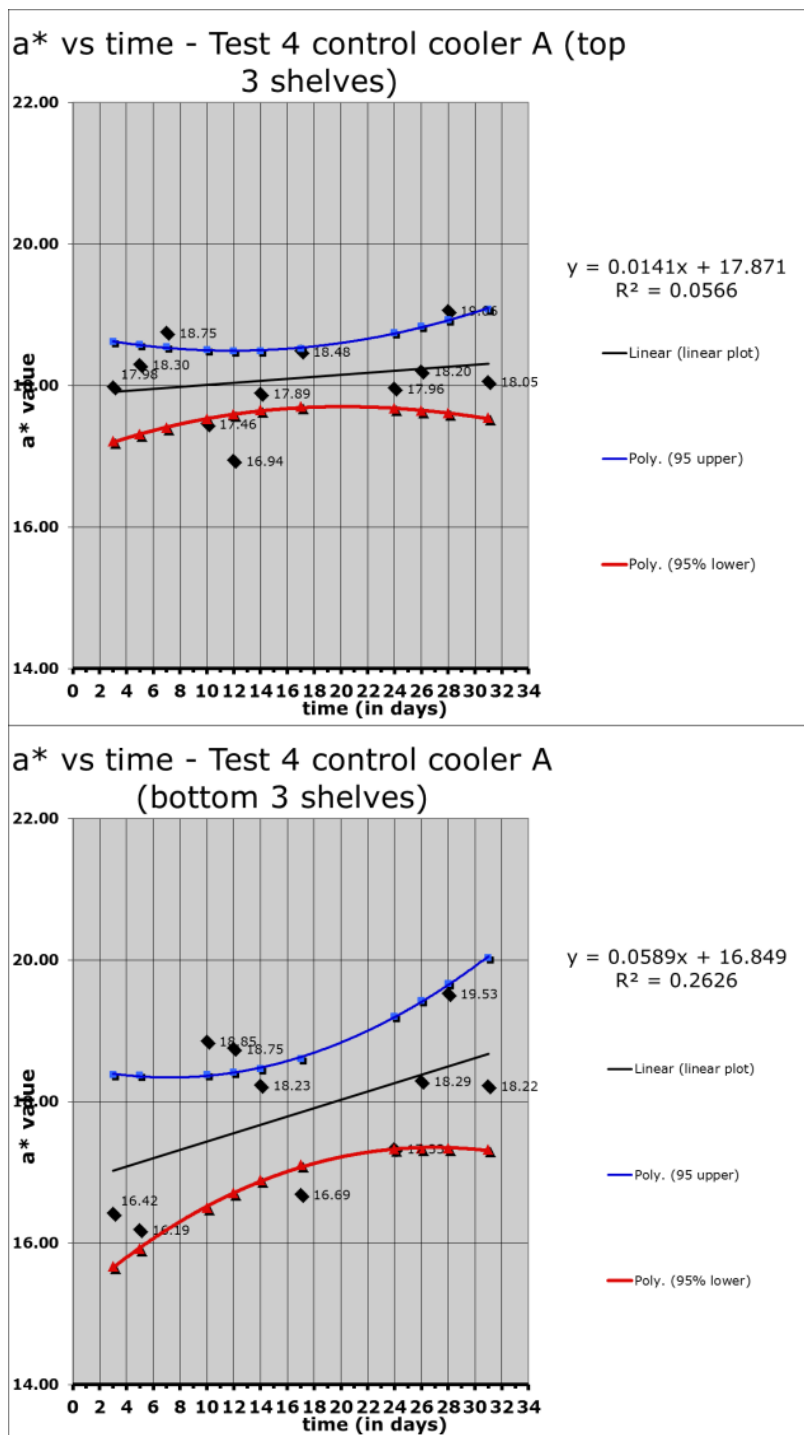
**Table 4.73** Test 4  $a^*$  values for both the control and scavenger samples over time with all data points included. Results are organized into top shelf and bottom shelf performance for both applications

day	Control cooler A	Control cooler A	Test cooler B	Test cooler B
	top three shelves $a^*$	bottom three shelves $a^*$	top three shelves $a^*$	bottom three shelves $a^*$
3	17.98	16.42	11.05	17.32
5	18.30	16.19	17.62	19.03
7	18.75	12.18	18.00	18.73
10	17.46	18.85	18.03	19.10
12	16.94	18.75	18.53	16.82
14	17.89	18.23	19.76	17.66
17	18.48	16.69	19.20	19.36
24	17.96	17.33	17.81	17.80
26	18.20	18.29	18.52	17.13
28	19.06	19.53	18.24	16.89
31	18.05	18.22	18.42	19.73
<b>ave.</b>	18.10	17.33	17.74	18.14
<b>min.</b>	16.94	12.18	11.05	16.82
<b>max</b>	19.06	19.53	19.76	19.73
<b>range</b>	2.12	7.35	8.71	2.91

Entering the  $a^*$  values from Table 4.73 above into the kinetics data input sheet (Tables 4.74 – 4.75) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).



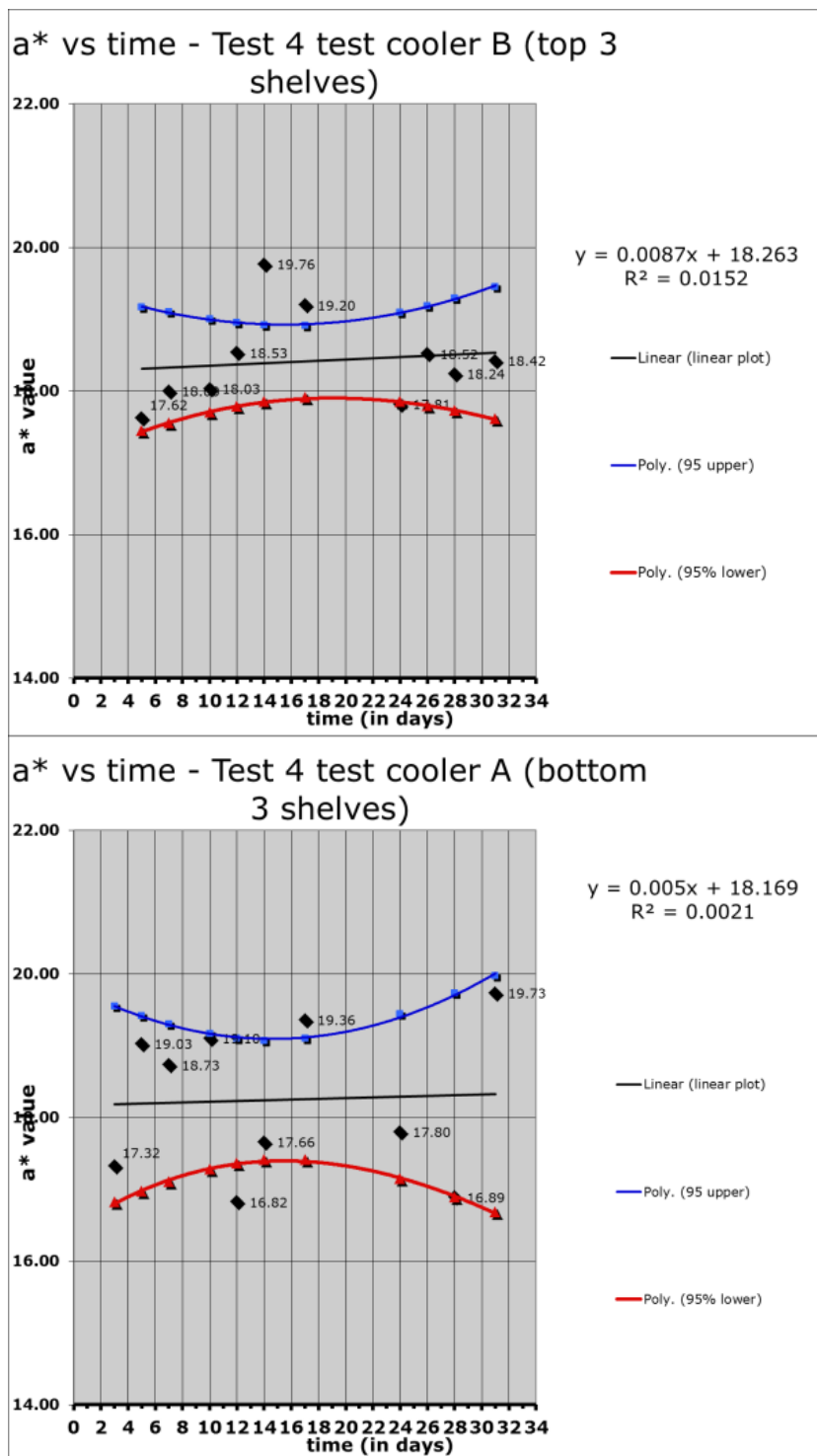




**Figure 4.51** A: Control Ham (top three shelves) Test 4 Zero order plot of  $a^*$  vs. time (31 days) with 95 % confidence limits calculation. B: Control Ham (bottom three shelves) test 4 Zero order plot of  $a^*$  vs. time (31 days) with 95% confidence limits calculation

**Table 4.75** Test 4  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the UV package A: top shelf only, B: Bottom shelf only

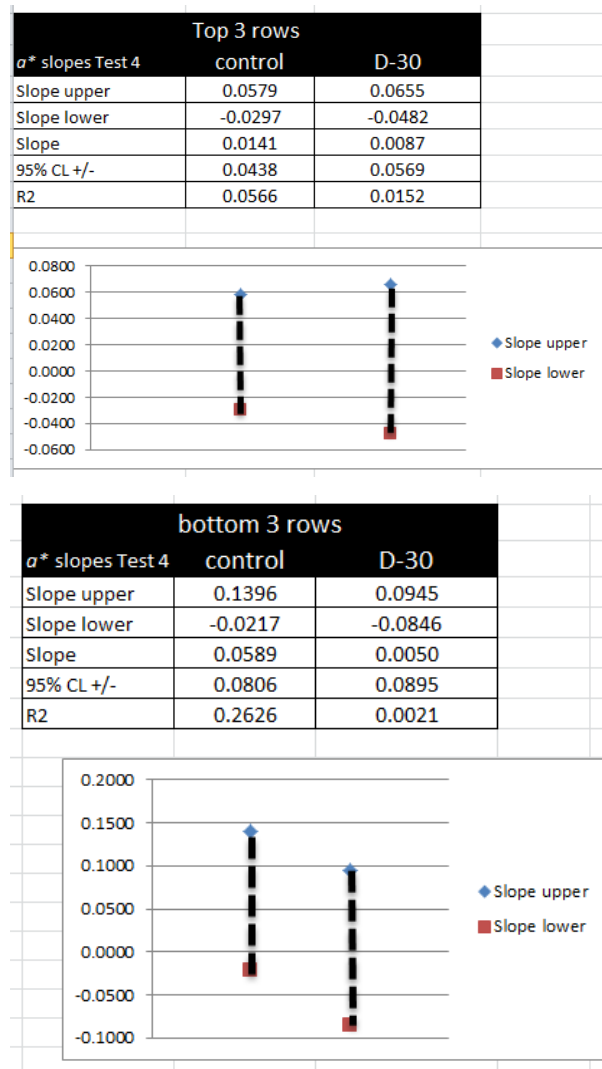
1. Raw Data:		Top row test test 4															
# data pairs Total=		10 This is automatically counted															
Y units		a*															
X units		days															
STATISTICS																	
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rd(yi-yes)*2																	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte		
	17.62	5	310.46	17.62	18.31	25.00	17.62	18.31	0.47	153.76	88.10	25.00	19.17	17.44	1.73	18.31	
	18.00	7	323.88	18.00	18.32	49.00	18.00	18.32	0.11	108.16	125.98	49.00	19.10	17.55	1.55	18.32	
	18.03	10	325.08	18.03	18.35	100.00	18.03	18.35	0.10	54.76	180.30	100.00	19.00	17.70	1.31	18.35	
	18.53	12	343.48	18.53	18.37	144.00	18.53	18.37	0.03	29.16	222.40	144.00	18.95	17.78	1.17	18.37	
	19.76	14	390.59	19.76	18.38	196.00	19.76	18.38	1.90	11.56	276.69	196.00	18.92	17.85	1.07	18.38	
	19.20	17	368.51	19.20	18.41	289.00	19.20	18.41	0.62	0.16	326.34	289.00	18.91	17.91	1.00	18.41	
	17.81	24	317.31	17.81	18.47	576.00	17.81	18.47	0.43	43.56	427.52	576.00	19.10	17.85	1.25	18.47	
	18.52	26	342.67	18.52	18.49	676.00	18.52	18.49	0.00	73.96	481.43	676.00	19.19	17.79	1.40	18.49	
	18.24	28	332.70	18.24	18.51	784.00	18.24	18.51	0.07	112.36	510.72	784.00	19.29	17.72	1.57	18.51	
	18.42	31	339.42	18.42	18.53	961.00	18.42	18.53	0.01	184.96	571.12	961.00	19.45	17.61	1.84	18.53	
			0.00	0.00	18.26	0.00	0.00	18.26	333.53	302.76	0.00	0.00	19.37	17.15	2.22	18.26	
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte	
				slope=		0.0087							Standard Er		0.68		
				intercept=		18.2628							Sum (yi-yes)		337.27		
				rsq=		0.0152							n		10.00		
				± 95% slope		0.0569							t 95%,2,n-2=		2.31		
				k upper		0.0655							x average =		17.40		
				k lower		-0.0482											
				Equations									Sum (xi-xav)		1075.16		
				Y = 18.2628		0.0087		* time					(Sum x)^2		30276.00		
													Sum(y^2)		3394.31		
													sum y		184.13		
													Sum (xi*yi)		3210.60		
													sum x		174.00		
													sum (X^2)		3800.00		
1. Raw Data:		Bottom row test - test 4															
# data pairs Total=		10 This is automatically counted															
Y units		a*															
X units		days															
STATISTICS																	
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rd(yi-yes)*2																	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte		
	17.32	3	300.10	17.32	18.18	9.00	17.32	18.18	0.74	146.41	51.97	9.00	19.55	16.82	2.73	18.18	
	19.03	5	362.01	19.03	18.19	25.00	19.03	18.19	0.69	102.01	95.13	25.00	19.42	16.97	2.45	18.19	
	18.73	7	350.94	18.73	18.20	49.00	18.73	18.20	0.28	65.61	131.13	49.00	19.30	17.10	2.20	18.20	
	19.10	10	364.94	19.10	18.22	100.00	19.10	18.22	0.78	26.01	191.03	100.00	19.16	17.27	1.89	18.22	
	16.82	12	283.02	16.82	18.23	144.00	16.82	18.23	1.98	9.61	201.88	144.00	19.10	17.36	1.74	18.23	
	17.66	14	311.88	17.66	18.24	196.00	17.66	18.24	0.34	1.21	247.24	196.00	19.07	17.41	1.67	18.24	
	19.36	17	374.68	19.36	18.25	289.00	19.36	18.25	1.22	3.61	329.06	289.00	19.10	17.41	1.69	18.25	
	17.80	24	316.84	17.80	18.29	576.00	17.80	18.29	0.24	79.21	427.20	576.00	19.44	17.14	2.30	18.29	
	16.89	28	285.27	16.89	18.31	784.00	16.89	18.31	2.01	166.41	472.92	784.00	19.73	16.89	2.84	18.31	
	19.73	31	389.27	19.73	18.32	961.00	19.73	18.32	1.98	252.81	611.63	961.00	19.97	16.68	3.29	18.32	
			0.00	0.00	18.17	0.00	0.00	18.17	330.12	228.01	0.00	0.00	19.75	16.58	3.17	18.17	
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte	
				slope=		0.0050							Standard Er		1.13		
				intercept=		18.1693							Sum (yi-yes)		340.38		
				rsq=		0.0021							n		10.00		
				± 95% slope		0.0895							t 95%,2,n-2=		2.31		
				k upper		0.0945							x average =		15.10		
				k lower		-0.0846											
				Equations									Sum (xi-xav)		1080.91		
				Y = 18.1693		0.0050		* time					(Sum x)^2		22801.00		
													Sum(y^2)		3338.95		
													sum y		182.45		
													Sum (xi*yi)		2759.20		
													sum x		151.00		
													sum (X^2)		3133.00		



**Figure 4.52 A:** Test (scavenger) Ham (top three shelves) Test 4 Zero order plot of  $a^*$  vs. time (31 days) with 95 % confidence limits calculation. **B:** Test (scavenger) Ham (bottom three shelves) Test 4 Zero order plot of  $a^*$  vs. time (31 days) with 95 % confidence limits calculation

Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments is provided in Table 4.76. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 4.76**  $a^*$  rate constant ( $k$ ) upper and lower for all applications in Test 4 as established by Labuza' Reaction kinetics shelf life model



Similar to Tests 1-3, the high variability of the  $a^*$  values over time resulted in a poor fit of data as measured by  $R^2$  (Table 4.76), and there was no statistical difference between the control samples and test samples with a scavenger. Some variability was also seen in performance between the top and bottom shelves within the same cooler (Figure 4.51 – 4.52), but not statistically significant. The line of best fit for both the control and scavenger sample were established near  $a^* = 18$  (Figures 4.51 -4.52). Explanations for these causes of this variability are discussed in Test 1.

#### 4.4.5 $L^*$ values Test 4

The  $L^*$  value average, minimum and maximum scores were also similar between control and scavenger samples (Table 4.77). Based on color score, the control package on the bottom three shelves had the darkest average score ( $L^* = 59.83$ ), while the test scavenger sample had the lightest average score ( $L^* = 59.01$ ). These results did not align with visual observations, which are discussed further in 4.4.5 below.

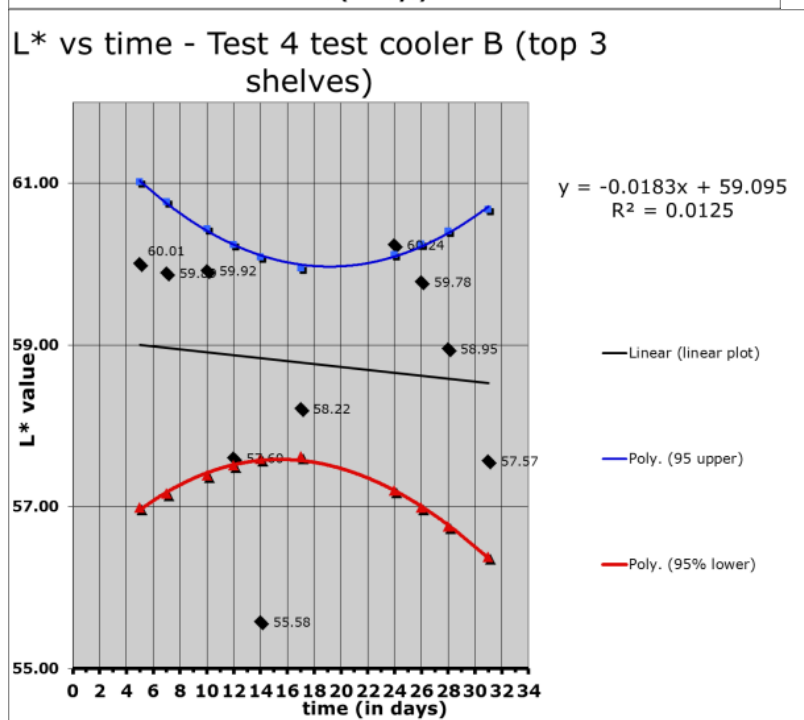
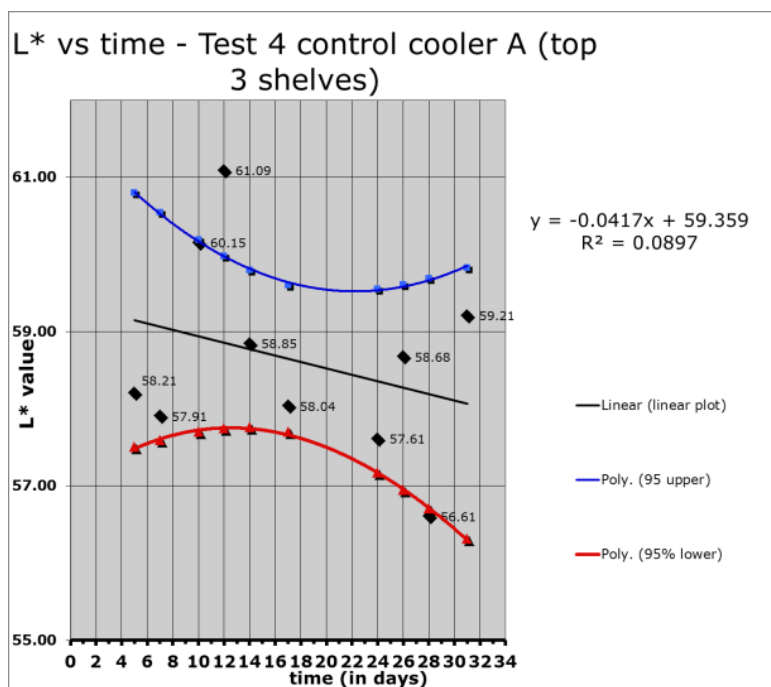
**Table 4.77**  $L^*$  value averages, minimum and maximum scores from Test 4. Test represents D-30 cc scavenging sachet

day	Control cooler A top three shelves $L^*$	Control cooler A bottom three shelves $L^*$	Test cooler B top three shelves $L^*$	Test cooler B bottom three shelves $L^*$
3	56.95	60.94	61.36	60.47
5	58.21	61.44	60.01	57.58
7	57.91	60.89	59.89	58.33
10	60.15	59.08	59.92	58.81
12	61.09	58.95	57.60	60.20
14	58.85	56.59	55.58	60.98
17	58.04	62.46	58.22	56.86
24	57.61	61.15	60.24	60.56
26	58.68	57.87	59.78	60.10
28	56.61	60.04	58.95	60.71
31	59.21	58.76	57.57	55.48
<b>ave.</b>	58.48	59.83	59.01	59.10
<b>min.</b>	56.61	56.59	55.58	55.48
<b>max</b>	61.09	62.46	61.36	60.98
<b>range</b>	4.48	5.87	5.78	5.50

Entering the  $L^*$  values from Table 4.77 above into the kinetics data input sheet (Table 4.78) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey or concentration of pigments (which is also an indication of moisture loss).



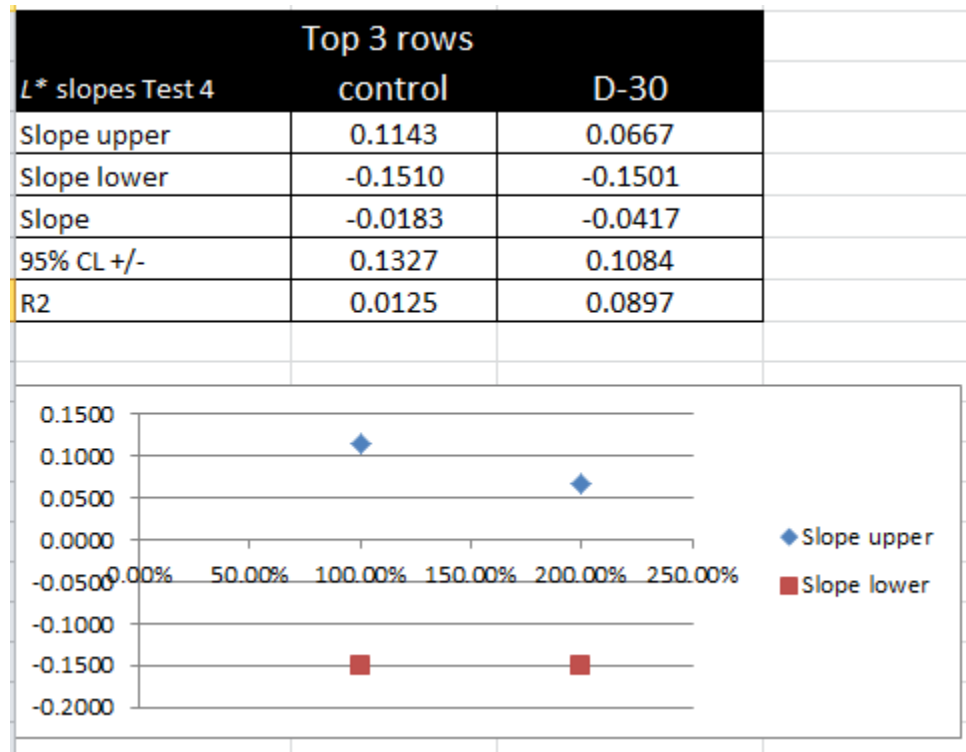




**Figure 4.53** A: Control (scavenger) Ham (top three shelves) Test 4 Zero order plot of  $L^*$  vs. time (31 days) with 95 % confidence limits calculation. B: Test (scavenger) Ham (top three shelves) Test 4 Zero order plot of  $L^*$  vs. time (31 days) with 95 % confidence limits calculation.

Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges (+k for lightening over the shelf life, - k for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.79.

**Table 4.79**  $L^*$  parameter rate constant (k) upper and lower for all applications in test 9 all lanes as established by Labuza' Reaction kinetics shelf life model.



There is no statistical difference in  $L^*$  value performance between the control and scavenger packaged sample for the top three shelves (Figure 4.53). Due to the high variability of values over time, the fit of the data is poor, and the line of best fit indicates a decrease (lightening) of  $L^*$  over time for both applications (Figure 4.53).

#### 4.4.6 Visual appearance of ham Test 4

During the first week, visual inspection of the ham was completed with packaging removed on days 3, 5 and 7. The only sandwiches that developed perceptible discoloration were those with oxygen levels above 19% (Appendix D.1 – D.3). Throughout the study, control and test sample number 20 were photographed in the package (Appendix D.4). The disadvantage of this method is oxygen content and color score are not established for these products, but a significant advantage is the same product is reviewed each time allowing for better insight on same sample color changes. Two observations from reviewing the photos of sample and control number 20 from day 3 to day 31 are 1) The control appears to be overall lighter in pink color intensity throughout the study compared to the scavenger sample which is darker pink and 2) The color from day to day for both control and scavenger does not appear to change significantly. The  $L^*$  values do not support a statistical difference in color lightness and darkness, but the color measurement method is not ideal as the lens of the chromameter only captures a small percentage of the surface area that has been affected by light. The visual appearance of the packaged sandwich provides evidence that the scavenger may be helping prevent a lighter “washed out” appearance from developing in the ham over time.

#### 4.4.7 Cooler temperatures Test 4

The average cooler temperatures are provided in Table 4.80. Temperature tracking charts are found in Appendix D.5.

**Table 4.80** Cooler temperatures Test 4

Test 4			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.36	-4.5	5.5
B	0.99	-2.5	5.5

#### 4.4.8 Conclusions Test 4

The predicted rate constants for both  $L^*$  and  $a^*$  values of the control and D-30 cc O<sub>2</sub> scavenging sachet are not statistically different. This corresponded however with the

absence of visual discoloration in all samples (both control and scavenger) with residual oxygen  $< 0.5\%$ . Although the scavenger produced lower residual oxygen packages compared to the control, the scavenger did not achieve 0% oxygen in all packages, indicating a potential need to explore a scavenger with greater capacity or better air flow around it in the package. Air flow around the scavenger is difficult to control as it is placed loose in the sandwich and can shift during transportation. There was no statistical difference over time with  $L^*$  values, but the visual appearance of the packaged control sandwich was lighter than the scavenger from day 3, which is an indication of fading. This lightness continued in the control packages throughout the 31 days. Sliced ham packaged alone with a 50 cc fast reacting oxygen scavenger has yielded positive results for color retention as measured by  $a^*$  value (Anderson and Rasmussen, 1992). There is visual evidence from this test that warrant continuing to explore an oxygen scavenger as a potential solution.

## **4.5 Test 5 –Use of ultraviolet (UV) films to control photooxidation, oxygen scavenger revisited**

### **4.5.1 – Overview of Test 5**

This test was designed to evaluate additional UV film combinations identified after Test 2 that might reduce ham color changes by limiting the amount of UV light the surface of the ham is exposed to. Photooxidation of nitrosylmyoglobin in a 20% CO<sub>2</sub> + 80% N<sub>2</sub> gas flush with residual oxygen levels of 0.1%, 0.5%, and 1.0% depends linearly on the amount of oxygen present in both the visible (436 nm) and UV spectrum (366 nm) (Møller, Bertelsen and Skibsted, 2002). Kampschmidt also demonstrated wavelengths of light between 400 and 550 nm to be an area in which cured meat absorbs light and brings about discoloration (Kampschmidt, 1955).

The previous UV film test (test 2) used Polyethylene terephthalate (PET) with an additive that blocks light at 380 nm using UV absorbing technology (UV light is absorbed by the film, not reflected or blocked). This film did not result in any significant reduction in meat discoloration compared to the control. For this Test 5, other combination films are explored. This includes combinations of the originally tested UV PET film with a PE layer that contains multiple additives with UV protecting properties.

The UV PET blocks from 350nm to 400nm, with a continuous reduction on the UV light blocking that is better than a regular non-UV PET film. At 360nm UV PET blocks 87% while non-UV PET blocks only 18% (5 times better). At 380nm UV PET blocks 42% while non-UV PET blocks only 17% (2.5 times better). At 400nm both UV PET and non-UV PET block 16%. (S.Utecht, personal communication, August 12, 2012) Using a UV additive in the PE (sealant) layer at the intended percentage (in a 2 mil film) blocks approximately 98% UV at 300 nm, 90% at 375 nm and almost 60% at 400 nm. A drawback to the addition of the additive in a 2 mil sealant film is that it potentially increases the hazing of the film appearance by a factor of two. There is also a cost associated with this technology, as a food safe option is required and there is limited demand for food safe UV films. The cost is approximately 20% higher than the control film (adding approximately \$.002 of cost per sandwich). (J.Vandeloo, personal

communication, November 25, 2014) Despite the potential of haze / increased distortion, the packaging appearance is primarily transparent, which makes it a viable consumer friendly option to consider.

An attempt was also made to re-test the Multisorb D-30 scavenger in this study.

#### **4.5.2 – Methods and Materials.**

Initially, six UV blocking test films were developed by Belmark<sup>®</sup> for consideration. The constructions of these films are 2-ply or 3-ply structures. A 2-ply structure is one with oriented PET (Polyethylene terephthalate) cast film is laminated (with adhesive) to a blown PE (Polyethylene) film. A 3- ply structure is one with oriented PET film laminated to a middle ply of oriented PET film laminated to a blown PE film.

The blown film extrusion process starts with resin pellets that are melted down and extruded out of a circular die, inflated several times, and formed into a thin film bubble. The bubble, cooled through the manufacturing process, is collapsed and slit into individual rolls of film. Blown film extrusion is the most widely used process for manufacturing sealant films. The term sealant as it is referenced below is synonymous with the LLDPE (linear low density polyethylene) or PE blown film layer. When a UV additive is placed in the sealant layer, it is referred to as “UVPE” or “UV sealant #” in the below charts. The UV inhibiting additives for this test are either in the PET or LLDPE layer (or both in some cases). The adhesive used to bind the layers is a polyester polyurethane solvent less adhesive system with EVOH located as a co-extrusion layer in the sealant.

In the previous UV film test (Test 2) it was determined that a UV additive to the PET layer alone did not add any benefit in reducing or eliminating the discoloration seen in the ham. With this test, two different UV inhibitor additives from different suppliers are explored in the blown PE layer (marked as UV sealant #1 and UV sealant #2). The additive is in resin form and is blended into the polyethylene (also in resin form) at the point the resins enter the hopper that feed the extruder.

UV sealant #1 and UV sealant #2 use different technologies to reduce exposure to light in the UV spectrum. UV sealant #1 uses a UV absorbing technology. This technology “screens” UV light from penetrating to the packaging content by absorbing the light into the molecular structure of the film. Using the additive in the sealant at the intended percentage (between 2 & 8% depending on caliper (thickness) of the sealant film and the amount of allowable haze) absorbs UV light. In a 2 millimeter caliper film, up to 8% addition of the additive will block approximately 98% of UV light at 300 nm (nanometers), 90% at 375 nm and almost 60% at 400 nm. With more additive, the film appearance becomes hazier.

UV sealant #2 used UV blocking technology. The additive functions by allowing visible light to pass through and preferentially scatter light in the UV spectrum. It is especially effective in blocking UV transmission in the 250-350nm range and will block about 80% of UV in that particular range.

With limited available space in refrigeration, the number of UV blocking films tested was reduced to three. The PET film with UV additive (referred to as “UV PET”) in the test films 2, 3, 5, & 6 below are all the same structure.

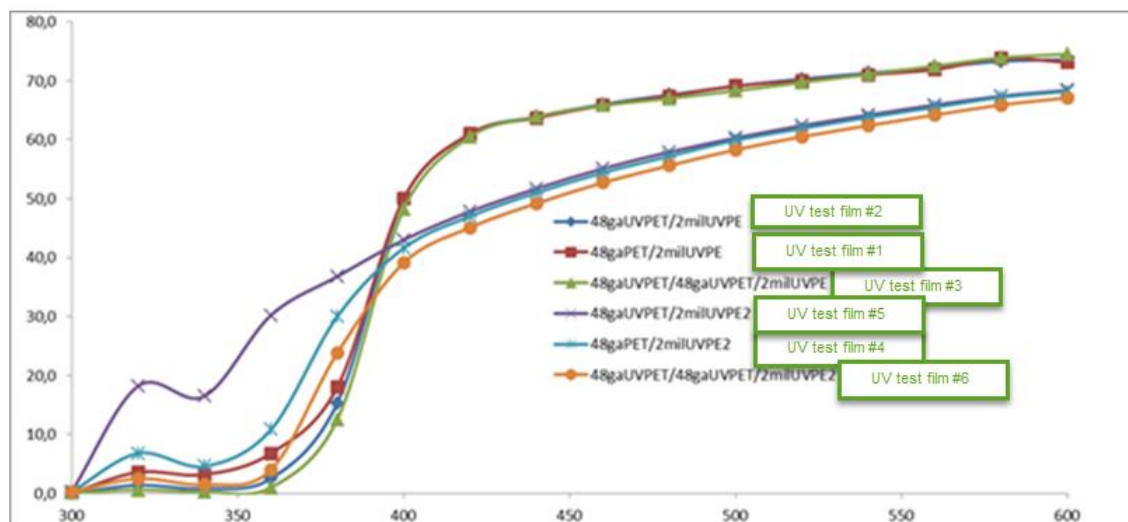
The initial Belmark<sup>®</sup> UV test films considered are listed below as 1 - 6. The films from this point on are referred to as numbered here (for example UV test film 3 refers to number 3 on this list). The OTR and WVTR are the same for all films (OTR 0.1469 cc/100in<sup>2</sup>/24 hours at 73°F / 0%RH; MVTR 0.4875 g/100in<sup>2</sup>/24 hours at 100°F / 90%RH).

- 1) UV test film 1 - PET/adhesive /UV sealant #1
- 2) UV test film 2 - UV PET/ adhesive / UV sealant #1 – **Not used in this study**
- 3) UV test film 3 - UV PET/ adhesive / UV PET/ adhesive / UV sealant #1
- 4) UV test film 4 - PET/ adhesive / UV sealant #2
- 5) UV test film 5 - UV PET/ adhesive /UV sealant #2 - **Not used in this study**

6) UV test film 6 - UV PET/ adhesive /UV PET/ adhesive / UV sealant #2 - **Not used in this study**

Films 1 and 4 were selected to establish the effectiveness of each UV additives to the LLDPE layer (sealant #1 & #2) alone. Test film 3 was selected to see if a combination of two UV hurdles (UV PET and UVPE) in all layers would perform better together, than separately.

Belmark provided a chart of the UV transmittance allowed for each of the original six variables recommended (As established using a UV-Visible spectrometer Hitachi U-2001 operating in transmission mode and scanning the sample from 200 to 600 nanometers, at 10 nm increments). The sealant film from supplier 1 allows less UV transmittance in the lower end of the spectrum while the UV supplier from supplier 2 allows less UV transmittance at the higher end of the spectrum (Figure 4.54).



**Figure 4.54** Chart of UV transmittance for test films submitted by Belmark®

All variables used in this test (including Belmark® UV films) are summarized in Table 4.81. The Belmark® UV films are marked as “1”, “3” and “4” in reference to the original 6 options considered.



**Table 4.81** Packaging materials used in test 5 (See section 3.2 & 3.5 for additional details)

Top films and materials used in test 5		
	Supplier	Functional addition for protecting meat discoloration
Control package (PET / adhesive / LLDPE)	Belmark®	no enhanced UV inhibiting or oxygen scavenging properties
UV blocking film #1: PET / adhesive / UV sealant #1	Belmark®	UV absorbing technology
UV blocking film #3: UV PET / adhesive / UV PET / adhesive / UV sealant #1	Belmark®	UV absorbing technology
UV blocking film #4: PET / adhesive / UV sealant #2	Belmark®	UV blocking technology
Control package (PET / adhesive / LLDPE) with D-30 Oxygen scavenger sachet placed in the pouch	Belmark® (film) Multisorb® (sachet)	Iron based oxygen scavenger

The bottom film used in all treatments is a black pigmented bottom forming film produced by Bemis® (Curwood) in Osh Kosh, WI. This film is a proprietary coextruded film with EVOH (Ethylene vinyl alcohol) as the active barrier to oxygen, polyester sealants & nylon structural layers. The starting thickness is 8 millimeter (mil), with a minimal thickness of 1 mil after forming. Barrier properties include oxygen <0.30 cc per 100 in<sup>2</sup> per 24 hours at 73°F and 0% RH (Relative humidity), WVTR <0.5 grams H<sub>2</sub>O per 100 in<sup>2</sup> per 24 hours at 100°F and 90% RH. All sandwiches had a label on the front of the package for retail sale. The label covers the upper 1/3 of the package, but leaves the lower 2/3 for exposure to light.

Three Beverage Air coolers (Model # LV27 c) with fluorescent lighting were used in this study. Each cooler contained two test variables (Table 4.82). Each shelf was filled one sandwich deep at the front position.

**Table 4.82** Cooler set up for test 5 Cooler A contained UV test film 1 (shaded in blue) and a control package (in white). Cooler B contained UV test film 3 (shaded in green) and a control package (in white). Cooler C contained UV test film 4 (in pink) and the D-30 Scavenger Sachet (in yellow). Numbers followed by the suffix “Pic” indicate samples that were photographed throughout the study and tested for oxygen%,  $a^*$  and  $L^*$  values on day 32

Date	10/24/2012						
<b>Cooler A</b>	1	2	3	4	5	6	7
Test Film 1 - PET/adh/UV sealant #1	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
CONTROL	C1	C2	C3	C4	C5	C6	C7 Pic
	C14	C13	C12	C11	C10	C9	C8
	C20	C19	C18	C17	C16	C15	
<b>Cooler B</b>	1	2	3	4	5	6	7
Test Film 3 - UV PET/adh/UV PET/adh/ UV sealant r #1	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
CONTROL	C21	C22	C23	C24	C25	C26	C27 Pic
	C28	C29	C30	C31	C32	C33	C34
	C35	C36	C37	C38	C39	C40	
<b>Cooler C</b>	1	2	3	4	5	6	7
Test Film 4 - PET/adh/UV sealant #2	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
Test Scavenger	S1	S2	S3	S4	S5	S6	S7 Pic
	S8	S9	S10	S11	S12	S13	S14
	S15	S16	S17	S18	S19	S20	

The sandwiches were evaluated twelve times throughout a thirty two day refrigerated shelf life for  $L^*a^*$  color, residual oxygen in the headspace and visual appearance out of the package using methods outlined in Test 2.

Method 1 of reconstructing bunched ham into full slices and flattening out) for  $L^*a^*$  color measurement was used. (Figure 3.9 in methods and materials)

Two sandwiches were removed from each cooler on selected days (Table 4.83) and products were evaluated for  $L^*a^*$  scores (raw data in Appendix E.8), oxygen percentage

in the headspace (raw data in Appendix E.8), and visual appearance out of the package (Appendix E.1-E.7).

**Table 4.83** Sandwich numbers evaluated and corresponding day in refrigerated shelf life in test 5

Day	Date	Cooler A	Cooler B	Cooler A	Cooler B	Cooler C	Cooler C
		Control # evaluated	Control # evaluated	UV test film 1 # evaluated	UV test film 3 # evaluated	UV test film 4 # evaluated	Oxygen scavenger sachet
Production	10/4/2012						
Day 1	10/12/2012						
Day 4	10/15/2012	6	26	7	7	7	
Day 6	10/17/2012	5	25	6	6	6	
Day 8	10/19/2012	4	24	5	5	5	
Day 11	10/22/2012	3	23	4	4	4	3
Day 13	10/24/2012	8	34	3	3	3	14
Day 15	10/26/2012	9	33	14	14	14	13
Day 20	10/31/2012	10	32	13	13	13	12
Day 22	11/2/2012	11	31	12	12	12	11
Day 25	11/5/2012	12	30	11	11	11	10
Day 27	11/7/2012	15	40	10	10	10	20
Day 29	11/9/2012	16	39	19	19	19	19
Day 32	11/12/2012	7	27	20	20	20	7

All sandwiches were assembled and put in MAP (Modified Atmosphere Packaging) with an 80% N<sub>2</sub> / 20% CO<sub>2</sub> blend at E.A. Sween Company using a Multivac R530. The ham, cheese and bread utilized are the same formulations used in test 1-4. All materials for each treatment are pulled from the same production lot codes to minimize variability. The ham and cheese was stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly. The bread was stored at room temperature (approximately 21° C) prior to assembly. The length of time from ham slicing to packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The sandwiches spent 7 days in dark frozen storage before the start of refrigerated shelf life.

### 4.5.3 – Oxygen percentages results for Test 5

The measured oxygen percentage in the package headspace is reported in Table 4.84.

**Table 4.84** Oxygen percentages over time in the headspace of the package during refrigerated shelf life in test 5. The scavenger sachet results are not reported for days 4, 6 and 8 due to the packages missing the sachet (which was not detected at the start due to the black bottom film).

	Cooler A	Cooler A	Cooler B	Cooler B	Cooler C	Cooler C
	Control film	UV test film 1	Control film	UV test film 3	UV test film 4	control film with Scavenger
day	oxygen %	oxygen %	oxygen %	oxygen %	oxygen %	oxygen %
4	0.44	0.39	0.35	0.43	0.34	not recorded
6	0.42	0.37	0.34	0.32	0.31	not recorded
8	0.40	0.31	0.34	0.36	0.40	not recorded
11	0.38	0.33	0.29	0.34	0.34	0.20
13	0.26	0.35	0.29	0.34	0.33	0.29
15	0.33	0.31	0.35	0.26	0.26	0.33
20	0.34	0.50	0.37	0.37	0.35	0.37
22	0.36	0.37	0.30	0.34	0.30	0.37
25	0.51	0.37	0.36	0.39	0.39	0.41
27	0.31	0.30	0.24	0.35	0.38	0.29
29	0.31	0.34	0.27	0.32	0.34	0.30
32	0.33	0.31	0.30	0.27	0.29	0.32
min	0.26	0.30	0.24	0.26	0.26	0.20
max	0.51	0.50	0.37	0.43	0.40	0.41
range	0.24	0.20	0.13	0.17	0.14	0.20

The range of O<sub>2</sub> % in the headspace throughout the 32 day study are similar for all treatments (0.20 – 0.51). This allows for a good comparison of results based on an important variable, i.e. oxygen, level in the package.

As discussed in the previous test 2, there are multiple potential causes for varied O<sub>2</sub> levels that occur during the three stages following sealing the package. The stages again are 1) levels immediately following manufacture, 2) post packaging release of trapped air followed by frozen storage, and 3) thaw followed by refrigerated storage (reviewed in test 2).

A key observation is that the oxygen percentage in the packages with the oxygen scavenger sachet were not at zero throughout the study ( $O_2$  % range of 0.2 - 0.41%) (Table 4.84 above), and are higher levels than observed in test 4 (0% - 0.064%). This may reinforce the finding that the scavenger does not have a large enough capacity or the capacity was depleted before going into the package (as the  $O_2$  levels were similar to the control). In the previous test 4, inclusion of the D-30 scavenger sachet also resulted in several packages that had available  $O_2$  in the headspace over the course of the shelf life, but 81% (18 of 22 packages) in test 4 did achieve 0% oxygen, where in this study no package with the scavenger over time achieved 0% oxygen. The scavenger's ability to remove  $O_2$  during the first 24 hours (before the freeze down is complete of the sandwich) is critical to its ability to slow meat discoloration. If the package is exposed to light prior to removal of  $O_2$ , photooxidation can occur.

#### **4.5.4 $-a^*$ scores for Test 5**

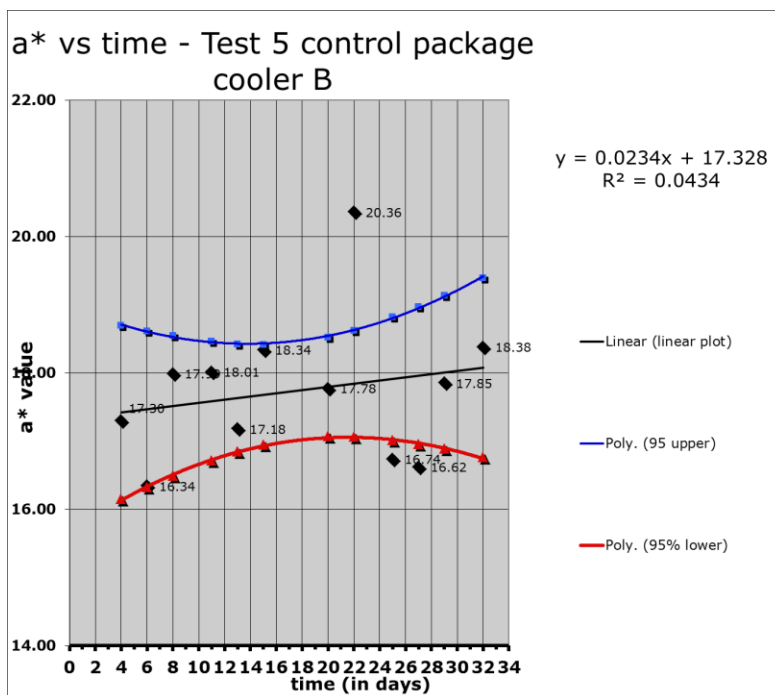
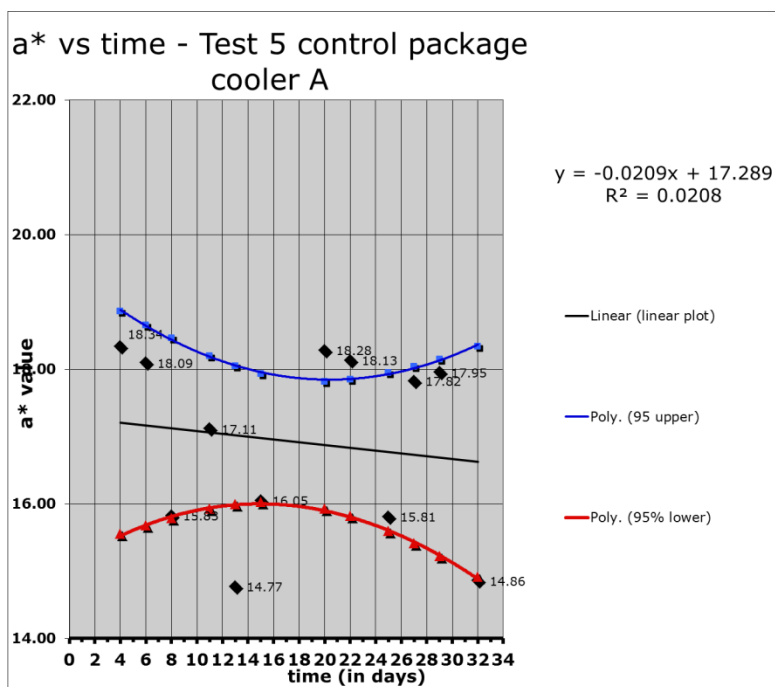
The variability of  $a^*$  values within and across all treatments over time was substantial with the range of 14.76 to 20.36 (Table 4.85). The minimum  $a^*$  values for each treatment occurred in lanes A and C at different days throughout the shelf life (highlighted in yellow in Table 4.85). The  $a^*$  values for the scavenger sachet for days 4 – 8 were discarded due to an error during processing (sachet missing).

**Table 4.85** Redness ( $a^*$ ) values over time for all treatments in test 5. Yellow highlights indicate the minimum value achieved for the treatment. The lowest  $a^*$  values were nearest the light source (Lanes A – C).

Day in shelf life	Cooler A Control Cooler A	Cooler A UV test film 1	Cooler B Control Cooler B	Cooler B UV test film 3	Cooler C UV test film 4	Cooler C Scavenger
	$a^*$	$a^*$	$a^*$	$a^*$	$a^*$	$a^*$
4	18.34	16.98	17.30	17.00	14.76	
6	18.09	17.09	16.34	17.14	15.90	
8	15.83	17.78	17.99	16.13	17.17	
11	17.11	19.28	18.01	17.42	17.67	18.40
13	14.77	18.94	17.18	17.36	18.68	17.13
15	16.05	18.92	18.34	15.14	16.33	18.81
20	18.28	16.83	17.78	17.14	17.56	16.70
22	18.13	19.52	20.36	17.27	18.84	18.12
25	15.81	18.58	16.74	16.71	17.16	18.86
27	17.82	18.83	16.62	18.75	18.34	16.89
29	17.95	18.99	17.85	17.28	16.92	18.96
32	14.86	16.99	18.38	17.02	16.76	17.10
<i>min</i>	14.77	16.83	16.34	15.14	14.76	16.70
<i>max</i>	18.34	19.52	20.36	18.75	18.84	18.96
<i>range</i>	3.57	2.69	4.02	3.61	4.07	2.26

Entering the  $a^*$  values from Table 4.85 above into the kinetics data input sheet (Tables 4.86 – 4.90) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

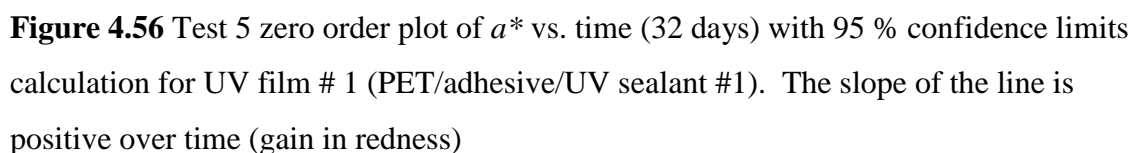




**Figure 4.55** Test 5 zero order plot of  $a^*$  vs. time (32 days) with 95 % confidence limits calculation. A: Control package cooler A. B: Control package cooler B. The slope of control package cooler A is negative over time (loss of redness). The slope of the control package in cooler B is positive over time (gain in redness)

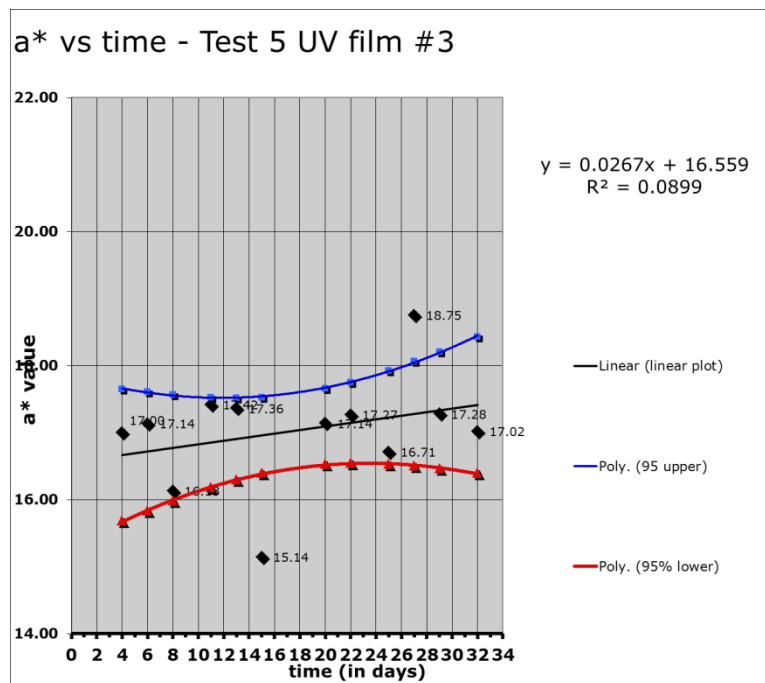


1. Raw Data:	UV test film 1																		
# data pairs Total=	12	This is automatically counted																	
Y units	a*																		
X units	days																		
STATISTICS																			
2. Calculati-Note after entering Y and X you need to pull down formulas in each column from top to last entry rd(yi-yes)*2																			
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average				
16.98	4	288.32	16.98	17.90	16.00	16.98	17.90	0.84	186.78	67.92	16.00	19.10	16.69	2.42	17.90				
17.09	6	292.07	17.09	17.94	36.00	17.09	17.94	0.73	136.11	102.54	36.00	19.03	16.86	2.18	17.94				
17.78	8	316.13	17.78	17.99	64.00	17.78	17.99	0.05	93.44	142.24	64.00	18.97	17.02	1.95	17.99				
19.28	11	371.59	19.28	18.07	121.00	19.28	18.07	1.47	44.44	212.04	121.00	18.89	17.24	1.66	18.07				
18.94	13	358.60	18.94	18.11	169.00	18.94	18.11	0.68	21.78	246.18	169.00	18.87	17.36	1.50	18.11				
18.92	15	357.84	18.92	18.16	225.00	18.92	18.16	0.57	7.11	283.75	225.00	18.86	17.47	1.39	18.16				
16.83	20	283.25	16.83	18.28	400.00	16.83	18.28	2.11	5.44	336.60	400.00	18.97	17.59	1.38	18.28				
19.52	22	381.03	19.52	18.33	484.00	19.52	18.33	1.41	18.78	429.44	484.00	19.07	17.59	1.48	18.33				
18.58	25	345.22	18.58	18.40	625.00	18.58	18.40	0.03	53.78	464.50	625.00	19.26	17.55	1.72	18.40				
18.83	27	354.69	18.83	18.45	729.00	18.83	18.45	0.14	87.11	508.50	729.00	19.41	17.49	1.92	18.45				
18.99	29	360.62	18.99	18.50	841.00	18.99	18.50	0.24	128.44	550.71	841.00	19.57	17.43	2.14	18.50				
16.99	32	288.66	16.99	18.57	1024.00	16.99	18.57	2.51	205.44	543.68	1024.00	19.82	17.33	2.50	18.57				
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average				
Equations																			
Y = 17.983    0.0243    * time																			



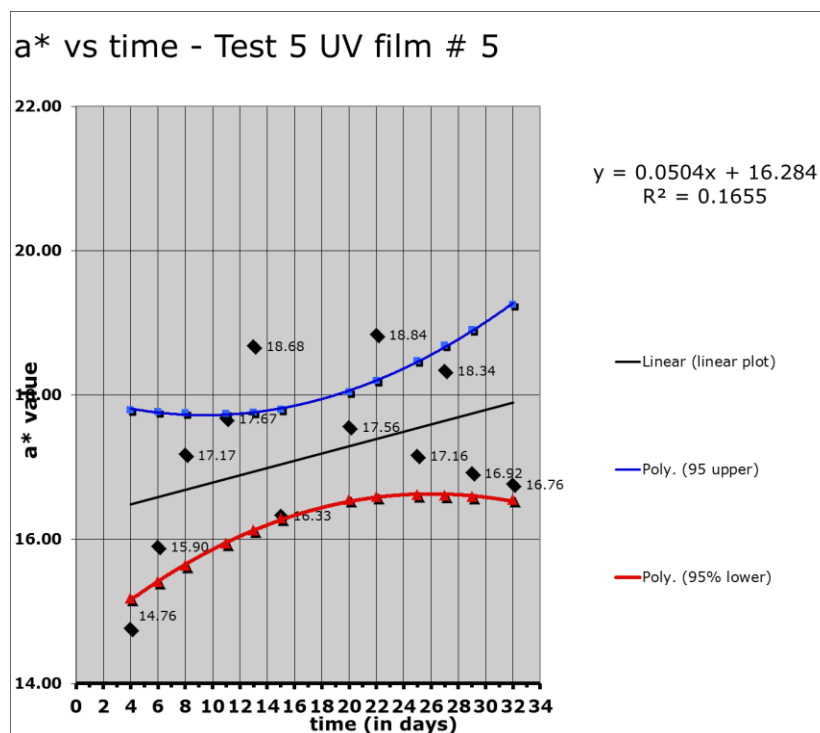
**Table 4.88** Test 5  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for test film 3 (UV PET/adhesive/UV PET/adhesive/ UV sealant #1)

1. Raw Data:			UV test film 3														
# data pairs Total=			12 This is automatically counted														
Y units			a*														
X units			days														
STATISTICS																	
2. Calculati																	
Note after entering Y and X you need to pull down formulas in each column from top to last entry of (y1-yes)^2																	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted	average
	17.00	4	289.11	17.00	16.67	16.00	17.00	16.67	0.11	186.78	68.01	16.00	17.65	15.68	1.97	16.67	
	17.14	6	293.67	17.14	16.72	36.00	17.14	16.72	0.17	136.11	102.82	36.00	17.60	15.83	1.77	16.72	
	16.13	8	260.18	16.13	16.77	64.00	16.13	16.77	0.41	93.44	129.04	64.00	17.57	15.98	1.59	16.77	
	17.42	11	303.46	17.42	16.85	121.00	17.42	16.85	0.32	44.44	191.62	121.00	17.53	16.18	1.35	16.85	
	17.36	13	301.49	17.36	16.91	169.00	17.36	16.91	0.21	21.78	225.72	169.00	17.52	16.29	1.22	16.91	
	15.14	15	229.32	15.14	16.96	225.00	15.14	16.96	3.30	7.11	227.15	225.00	17.53	16.39	1.13	16.96	
	17.14	20	293.78	17.14	17.09	400.00	17.14	17.09	0.00	5.44	342.88	400.00	17.65	16.53	1.12	17.09	
	17.27	22	298.14	17.27	17.15	484.00	17.27	17.15	0.01	18.78	379.87	484.00	17.75	16.54	1.21	17.15	
	16.71	25	279.22	16.71	17.23	625.00	16.71	17.23	0.27	53.78	417.75	625.00	17.93	16.53	1.40	17.23	
	18.75	27	351.69	18.75	17.28	729.00	18.75	17.28	2.17	87.11	506.34	729.00	18.06	16.50	1.56	17.28	
	17.28	29	298.60	17.28	17.33	841.00	17.28	17.33	0.00	128.44	501.12	841.00	18.20	16.46	1.74	17.33	
	17.02	32	289.68	17.02	17.41	1024.00	17.02	17.41	0.15	205.44	544.64	1024.00	18.43	16.40	2.03	17.41	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted	average
	slope=		0.0267														
	intercept=		16.5587														
	rsq=		0.0899														
	± 95% slope		0.0599														
	k upper		0.0866														
	k lower		-0.0332														
	Equations																
	Y =		16.5587		0.0267		* time										
	STATISTICS																
	Standard Error		0.85														
	Sum (yi-yes)		555.52														
	n		12.00														
	t 95%,2,n-2=		2.23														
	x average =		17.67														
	Sum (xi-xav)		1612.89														
	(Sum x)^2		44944.00														
	Sum(y^2)		3488.33														
	sum y		204.37														
	Sum (xi*yi)		3636.88														
	sum x		212.00														
	sum (X^2)		4734.00														



**Figure 4.57** Test 5 zero order plot of  $a^*$  vs. time (32 days) with 95 % confidence limits calculation for UV film # 3 (UV PET/adhesive/UV PET/adhesive/ UV sealant #1). The slope of the line is positive over time (gain in redness)

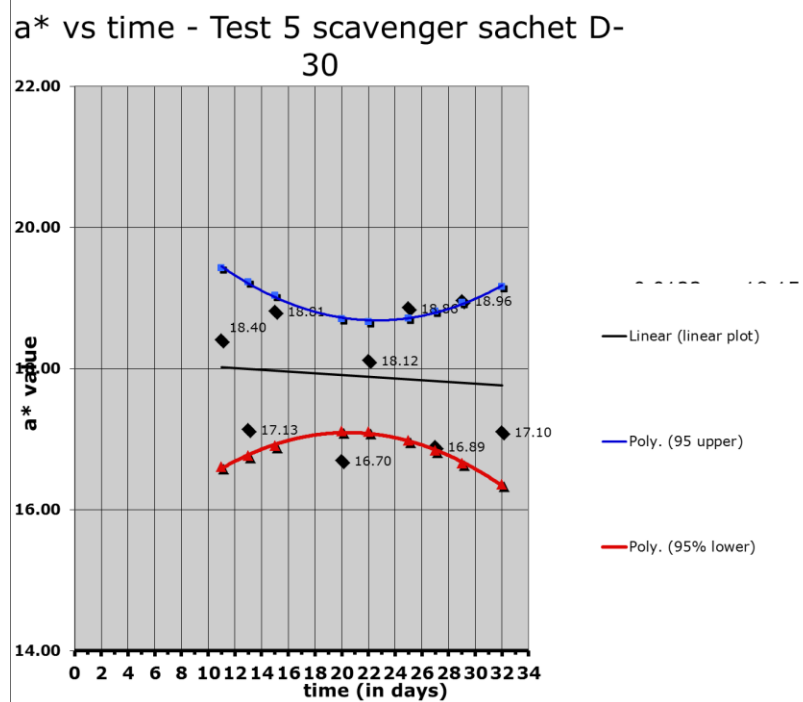
1. Raw Data:	UV film 4																		
# data pairs Total=	12	This is automatically counted																	
Y units	a*																		
X units	days																		
STATISTICS																			
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yes)²2 (xi-xave)²2xi*yi X²2 y 95%JL y 95%L Delta predicte																			
Y value	x= time	Y²2	Y plot value	Est yi	time²2	yi	yi estimate	yi-yes)²2	(xi-xave)²2xi*yi	X²2	y 95%JL	y 95%L	Delta	predicte					
14.76	4	217.96	14.76	16.49	16.00	14.76	16.49	2.96	186.78	59.05	16.00	17.79	15.18	2.62	16.49				
15.90	6	252.70	15.90	16.59	36.00	15.90	16.59	0.47	136.11	95.38	36.00	17.77	15.41	2.36	16.59				
17.17	8	294.92	17.17	16.69	64.00	17.17	16.69	0.24	93.44	137.39	64.00	17.74	15.63	2.12	16.69				
17.67	11	312.35	17.67	16.84	121.00	17.67	16.84	0.70	44.44	194.41	121.00	17.74	15.94	1.80	16.84				
18.68	13	348.82	18.68	16.94	169.00	18.68	16.94	3.02	21.78	242.80	169.00	17.75	16.12	1.63	16.94				
16.33	15	266.67	16.33	17.04	225.00	16.33	17.04	0.50	7.11	244.95	225.00	17.79	16.28	1.51	17.04				
17.56	20	308.24	17.56	17.29	400.00	17.56	17.29	0.07	5.44	351.13	400.00	18.04	16.54	1.50	17.29				
18.94	22	354.82	18.94	17.39	484.00	18.94	17.39	2.09	16.78	414.41	484.00	18.19	16.59	1.60	17.39				
17.16	25	294.47	17.16	17.54	625.00	17.16	17.54	0.15	53.78	429.00	625.00	18.47	16.81	1.86	17.54				
18.34	27	336.23	18.34	17.64	729.00	18.34	17.64	0.46	87.11	495.09	729.00	18.69	16.81	2.08	17.64				
16.92	29	286.17	16.92	17.74	841.00	16.92	17.74	0.69	128.44	490.58	841.00	18.90	16.59	2.32	17.74				
16.76	32	281.01	16.76	17.90	1024.00	16.76	17.90	1.28	205.44	536.43	1024.00	19.25	16.54	2.71	17.90				
Y value	x=time	Y²2	Y plot value	Est yi	time²2	yi	yi estimate	yi-yes)²2	(xi-xave)²2xi*yi	X²2	y 95%JL	y 95%L	Delta	predicte	average				



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**Table 4.90** Test 5  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for scavenger sachet D-30

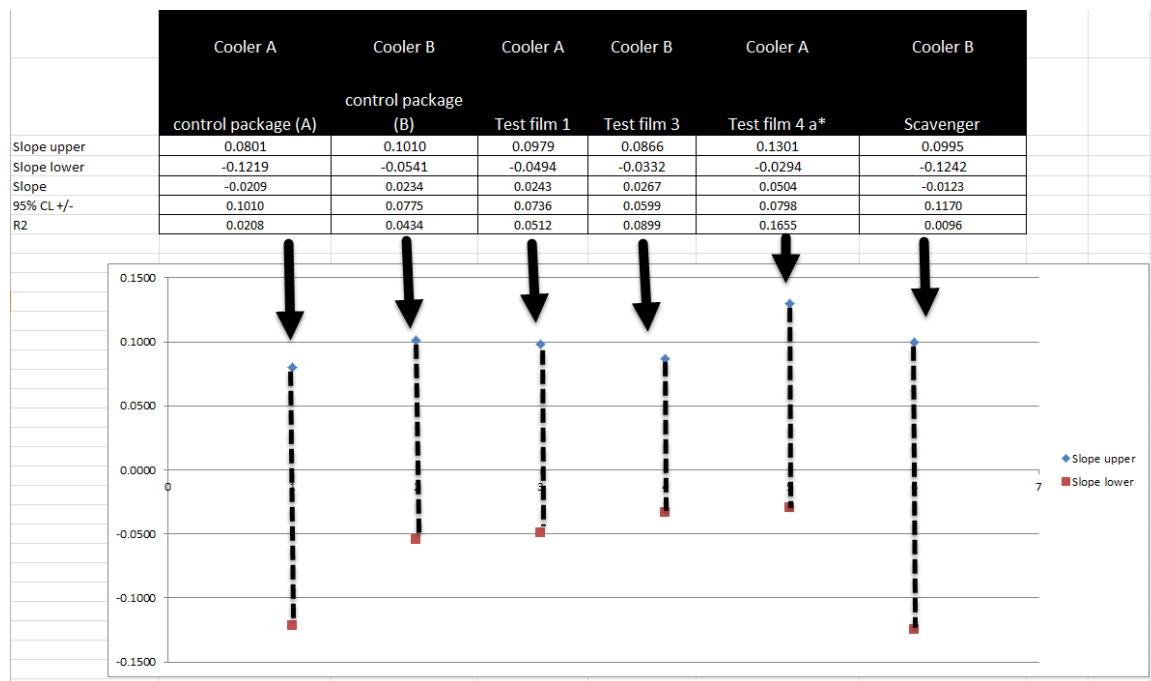
1. Raw Data:		Test 5 scavenger														
# data pairs Total=		9 This is automatically counted														
Y units		a*														
X units		days														
STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rd(yi-yes)*2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	18.40	11	338.56	18.40	18.02	121.00	18.40	18.02	0.15	111.42	202.40	121.00	19.43	16.60	2.83	18.02
	17.13	13	293.55	17.13	17.99	169.00	17.13	17.99	0.74	73.20	222.73	169.00	19.22	16.76	2.46	17.99
	18.81	15	353.94	18.81	17.97	225.00	18.81	17.97	0.72	42.98	282.20	225.00	19.03	16.90	2.14	17.97
	16.70	20	278.78	16.70	17.91	400.00	16.70	17.91	1.46	2.42	333.93	400.00	18.70	17.11	1.59	17.91
	18.12	22	328.21	18.12	17.88	484.00	18.12	17.88	0.06	0.20	398.57	484.00	18.66	17.10	1.56	17.88
	18.86	25	355.57	18.86	17.84	625.00	18.86	17.84	1.03	11.86	471.42	625.00	18.71	16.98	1.74	17.84
	16.89	27	285.38	16.89	17.82	729.00	16.89	17.82	0.86	29.64	456.12	729.00	18.81	16.83	1.97	17.82
	18.96	29	359.48	18.96	17.79	841.00	18.96	17.79	1.36	55.42	549.84	841.00	18.93	16.66	2.28	17.79
	17.10	32	292.52	17.10	17.76	1024.00	17.10	17.76	0.43	109.09	547.31	1024.00	19.16	16.36	2.80	17.76
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	slope=												Standard Error	0.98		
	intercept=												Sum (yi-yes)	1653.98		
	rsq=												n	9.00		
	± 95% slope												t 95%, 2, n-2=	2.37		
	k upper												x average =	21.56		
	k lower															
	Equations												Sum (xi-xav)	2759.43		
	Y = 18.1504 - 0.0123 * time												(Sum x)^2	37636.00		
													Sum(y^2)	2886.01		
													sum y	160.97		
													Sum (xi*yi)	3464.52		
													sum x	194.00		
													sum (X^2)	4618.00		



**Figure 4.59** Test 5 zero order plot of  $a^*$  vs. time (32 days) with 95 % confidence limits calculation for scavenger sachet D-30. The slope of the line is negative over time (loss in redness)

Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the slope ranges ( $+k$  for increase in  $a^*$  value (redness) over the shelf life,  $-k$  for loss of redness or decreasing  $a^*$  value at  $\pm$  95% CL) between treatments is provided in Table 4.91.

**Table 4.91**  $a^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in test 5 as established by Labuza' Reaction kinetics shelf life model.



While the slope of the line predicts a negative trend (loss of redness over time) for control package A and the oxygen scavenger treatment, and positive trend (gain in redness over time) for control package B and all UV film treatments; statistically, there is no difference in  $a^*$  color scores over a 32 day period for any of the treatments as at the 95% confidence level there is overlap in outcomes for all treatments (Table 4.91 above). The reasons for the difference between the two controls in cooler A and B is not clear, but at

day 32, the control package in cooler A had a much lower  $a^*$  score than the control in cooler B which added to the larger range for the control A package. At day 32, the sample in cooler A achieved  $a^* = 14.86$ , while cooler B achieved  $a^* = 18.38$ . Both sandwiches are in lane A in the same position in the cooler (shelf 4 with a similar distance from the light source) and have similar  $O_2$  levels (0.33% and 0.30%). While the  $a^*$  ranges are similar for both controls (3.57 for cooler A and 4.02 for cooler B), the cooler B sample achieves a higher minimum and maximum  $a^*$  value than the cooler A control (Table 4.85 above), which could be the result of variation with the starting ham color. Previous tests have found that the color variation at the time of slicing within a single batch have a range as large as 5.74 points (minimum = 14.25, Maximum = 19.98 – see section 3.11)).

Other potential causes for the difference between control samples over time include differences in light intensity exposure. As the angle on the shelf varies, so does the light intensity reaching the surface of the sandwich (as much as 1000 lux – see Methods and materials section 3.16). Even with the sandwiches in near identical locations from the light source, the angle of the sandwich on the shelf can reduce the intensity of the light reaching the surface of the ham.

It was noted in this study that the lowest  $a^*$  achieved occurred in lanes A – C. This is consistent with the previous tests 1-4. Literature regarding the impact of light source and intensity on meat color is inconsistent. Many of the studies agree that discoloration is proportional to light level and exposure time, but there is no consistency on whether ultraviolet (UV) or the visible spectrum is more damaging (Sylvania, 2014). In a study of sliced cooked cured ham in vacuum packaging, Li et al. found that illumination had no significant effect on the  $a^*$  value across the conditions of 1000, 200, and 0 lux through 28 days. The  $a^*$  values in this study varied, increasing during days 1-7 of storage and then decreasing over days 7-14 in all three lumination conditions. The differences in  $a^*$  value between lumination conditions wasn't significant (Li et al., 2012).

In regards to the performance differences between the controls, it is also possible that the control cooler A sample had a higher  $O_2$  level at the start of shelf life, but decreased to 0.33% by day 32. If greater oxygen was initially available, the potential for metmyoglobin formation over time would be greater. This is however unlikely given the

products were all assembled and packaged together within a 30 minute time frame, and the consistency of oxygen levels at day 32 was similar across all treatments.

The performance of UV test films 1, 3, and 4 were all similar (Table 4.91 above). While the statistical modeling would suggest that the UV films have a greater likelihood of a positive slope over time, they also have the potential for a negative slope, and the performance of all of the UV films was similar to the control package in cooler B. This leads to the conclusion that each UV additive alone or in combination was equally ineffective for slowing meat discoloration compared to the control package on the attribute of redness ( $a^*$ ).

#### 4.5.5 – $L^*$ scores for Test 5

The range of  $L^*$  values is similar for all treatments with the exception of the scavenger sachet package which was missing week 1 data (Table 4.92). UV test film 1 produced the lowest minimum  $L^*$  value (52.70) and lowest maximum value (60.21) while UV test film 4 produced the highest minimum (58.83) and highest maximum (66.70)  $L^*$  scores over time (Table 4.92).

**Table 4.92** Ham  $L^*$  values in test 5 for all treatments. Yellow highlight indicates the highest  $L^*$  achieved within each treatment (which is an indication of greatest amount of fade)

	Cooler A Control	Cooler A Control	Cooler B Control	Cooler B Control	Cooler C Control	Cooler C Control
Day in shelf life	Cooler A $L^*$	Test film 1 $L^*$	Cooler B $L^*$	Test film 3 $L^*$	Test film 4 $L^*$	Scavenger $L^*$
4	57.50	59.17	61.24	62.27	64.37	
6	61.07	60.19	64.34	60.66	66.70	
8	63.84	59.96	60.35	63.87	60.07	
11	61.61	60.21	60.14	60.62	61.86	58.72
13	62.71	58.96	59.20	60.86	60.22	61.54
15	62.15	52.70	58.12	60.76	59.60	57.69
20	59.30	59.20	57.88	58.50	58.83	61.05
22	60.09	56.64	57.63	60.00	60.26	60.2
25	63.86	58.53	62.36	62.67	61.11	59.5
27	58.15	59.22	60.82	60.10	59.30	58.49
29	59.88	56.64	57.61	62.27	59.82	57.47
32	60.76	58.43	56.71	57.65	59.28	57.98
<i>min</i>	57.50	52.70	56.71	57.65	58.83	57.47
<i>max</i>	63.86	60.21	64.34	63.87	66.70	61.54
<i>range</i>	6.37	7.52	7.63	6.22	7.87	4.07

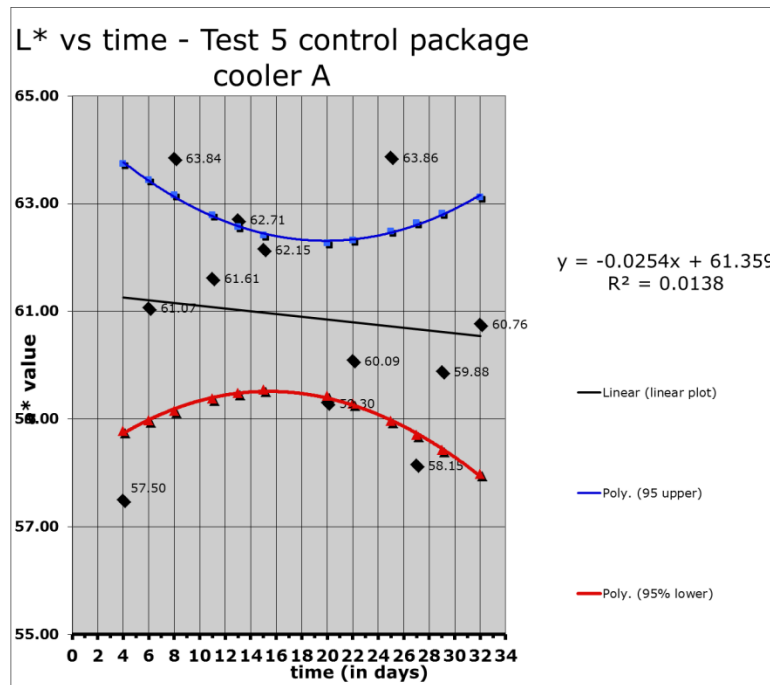
The differences in the ranges could be due to the differences in wavelengths absorbed or blocked between PE sealant #1 and #2 noted above with UV sealant #1 being more effective at 300-375 nm, and UV sealant #2 being more effective in the range of 250-350 nm. The highest  $L^*$  values achieved for each treatment occurred between days 4 – 13 in Lanes A – D. In contrast, the  $a^*$  value lows were achieved in lanes A – C between days 4-20. This is an indication that  $a^*$  and  $L^*$  outcomes often do not correlate for same day performance. A product could perform differently on  $a^*$  value and  $L^*$  value on a same day evaluation. This creates the potential for the product to be rejected by consumers for different attribute changes over a broader number of days during the refrigerated shelf life.

Entering the  $L^*$  values from Table 4.92 above into the kinetics data input sheet (Tables 4.93 – 4.98) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (also interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. In this study, a desired outcome would be no change over time. Lightening of the product over time could be interpreted as fade; however a darkening of the product could be an indication of formation of grey.



**Table 4.93** Test 5  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL control cooler A

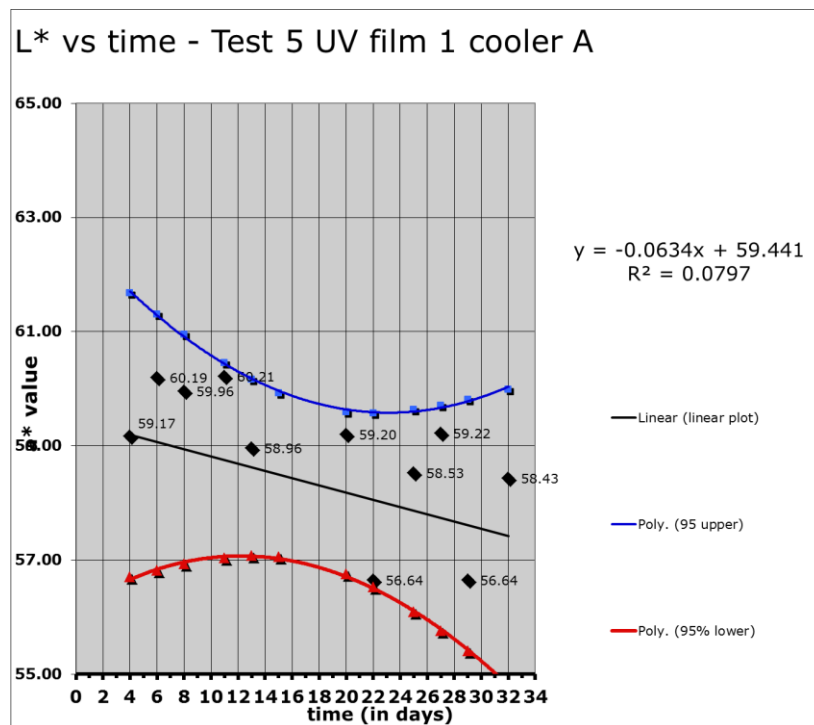
1. Raw Data:		Test 5 control cooler A																	
# data pairs		Total=		12 This is automatically counted															
Y units		L*																	
X units		days																	
STATISTICS																			
2. Calculati																			
Note after entering Y and X you need to pull down formulas in each column from top to last entry of		(y1-yes)^2 (xi-xave)^2 xi*Yi X^2 y 95%UL y 95%LL Delta predicted average																	
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average				
57.50	4	3305.87	57.50	61.26	16.00	57.50	61.26	14.14	186.78	229.99	16.00	63.74	58.77	4.97	61.26				
61.07	6	3729.14	61.07	61.21	36.00	61.07	61.21	0.02	136.11	366.40	36.00	63.45	58.97	4.48	61.21				
63.84	8	4075.97	63.84	61.16	64.00	63.84	61.16	7.23	93.44	510.75	64.00	63.16	59.15	4.02	61.16				
61.61	11	3796.20	61.61	61.08	121.00	61.61	61.08	0.29	44.44	677.75	121.00	62.79	59.37	3.41	61.08				
62.71	13	3932.13	62.71	61.03	169.00	62.71	61.03	2.82	21.78	815.19	169.00	62.57	59.48	3.09	61.03				
62.15	15	3862.62	62.15	60.98	225.00	62.15	60.98	1.37	7.11	932.25	225.00	62.41	59.54	2.87	60.98				
59.30	20	3516.09	59.30	60.85	400.00	59.30	60.85	2.41	5.44	1185.93	400.00	62.27	59.43	2.84	60.85				
60.09	22	3610.41	60.09	60.80	484.00	60.09	60.80	0.51	18.78	1321.91	484.00	62.32	59.28	3.05	60.80				
63.86	25	4078.53	63.86	60.72	625.00	63.86	60.72	9.86	53.78	1596.58	625.00	62.49	58.96	3.54	60.72				
58.15	27	3381.03	58.15	60.67	729.00	58.15	60.67	6.38	87.11	1569.96	729.00	62.64	58.70	3.94	60.67				
59.88	29	3586.01	59.88	60.62	841.00	59.88	60.62	0.55	128.44	1736.62	841.00	62.82	58.42	4.40	60.62				
60.76	32	3692.18	60.76	60.55	1024.00	60.76	60.55	0.05	205.44	1944.43	1024.00	63.12	57.98	5.14	60.55				
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average				
slope=				-0.0254												Standard Error		2.14	
intercept=				61.3586												Sum (yi-yes)		7575.36	
rsq=				0.0138												n		12.00	
± 95% slope				0.1515												t 95%,2,n-2=		2.23	
k upper				0.1261												x average =		17.67	
k lower				-0.1769															
Equations																Sum (xi-xav)		1612.89	
																(Sum x)^2		44944.00	
Y = 61.3586 -0.0254 * time																Sum(y^2)		44566.19	
																sum y		730.92	
																Sum (xi*yi)		12887.74	
																sum x		212.00	
																sum (X^2)		4734.00	



**Figure 4.60** Test 5 zero order plot of  $L^*$  vs. time (32 days) with 95 % confidence limits calculation for control cooler A

**Table 4.94** Test 5  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL UV test film 1 cooler A

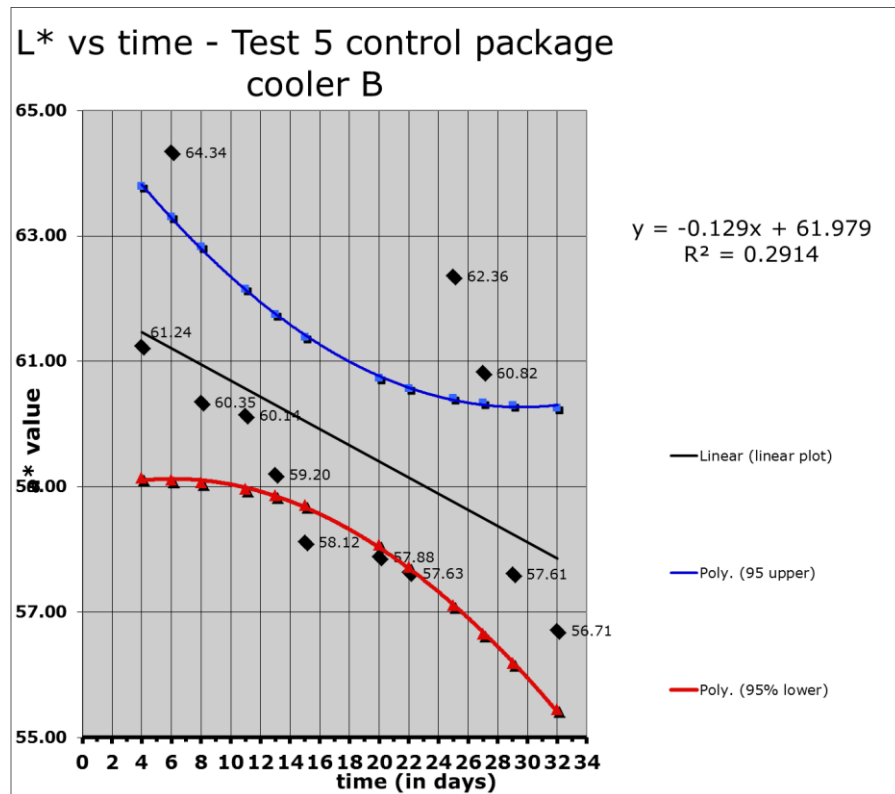
1. Raw Data:		Test 5 UV film 1															
# data pairs	Total=	12	This is automatically counted														
Y units	L*																
X units	days																
STATISTICS																	
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry of (y-yes)^2 (xi-xave)^2 xi*yi X^2 y 95%UL y 95%LL Delta predicted average																	
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted	average	
59.17	4	3501.09	59.17	59.19	16.00	59.17	59.19	0.00	186.78	236.68	16.00	61.68	56.70	4.98	59.19		
60.19	6	3623.24	60.19	59.06	36.00	60.19	59.06	1.28	136.11	361.16	36.00	61.31	56.82	4.49	59.06		
59.96	8	3595.20	59.96	58.93	64.00	59.96	58.93	1.05	93.44	479.68	64.00	60.95	56.92	4.03	58.93		
60.21	11	3625.65	60.21	58.74	121.00	60.21	58.74	2.16	44.44	662.35	121.00	60.45	57.03	3.42	58.74		
58.96	13	3476.67	58.96	58.62	169.00	58.96	58.62	0.12	21.78	766.52	169.00	60.17	57.07	3.10	58.62		
52.70	15	2776.94	52.70	58.49	225.00	52.70	58.49	33.57	7.11	790.45	225.00	59.93	57.05	2.87	58.49		
59.20	20	3504.25	59.20	58.17	400.00	59.20	58.17	1.05	5.44	1183.93	400.00	59.60	56.75	2.85	58.17		
56.64	22	3208.47	56.64	58.05	484.00	56.64	58.05	1.97	18.78	1246.15	484.00	59.57	56.52	3.05	58.05		
58.53	25	3425.37	58.53	57.86	625.00	58.53	57.86	0.45	53.78	1463.17	625.00	59.63	56.09	3.54	57.86		
59.22	27	3507.01	59.22	57.73	729.00	59.22	57.73	2.22	87.11	1598.94	729.00	59.71	55.75	3.95	57.73		
56.64	29	3208.47	56.64	57.60	841.00	56.64	57.60	0.92	128.44	1642.66	841.00	59.81	55.40	4.41	57.60		
58.43	32	3414.45	58.43	57.41	1024.00	58.43	57.41	1.04	205.44	1869.87	1024.00	59.99	54.84	5.15	57.41		
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted	average	
slope=												Standard Error		2.14			
intercept=												Sum (yi-yes)		7112.28			
rsq=												n		12.00			
± 95% slope												t 95%,2,n-2=		2.23			
k upper												x average =		17.67			
k lower																	
Equations																	
Y = 59.4409 -0.0634 * time																	
Sum (xi-xav)												1612.89					
(Sum x)^2												44944.00					
Sum (y^2)												40866.80					
sum y												699.86					
Sum (xi*yi)												12301.56					
sum x												212.00					
sum (X^2)												4734.00					



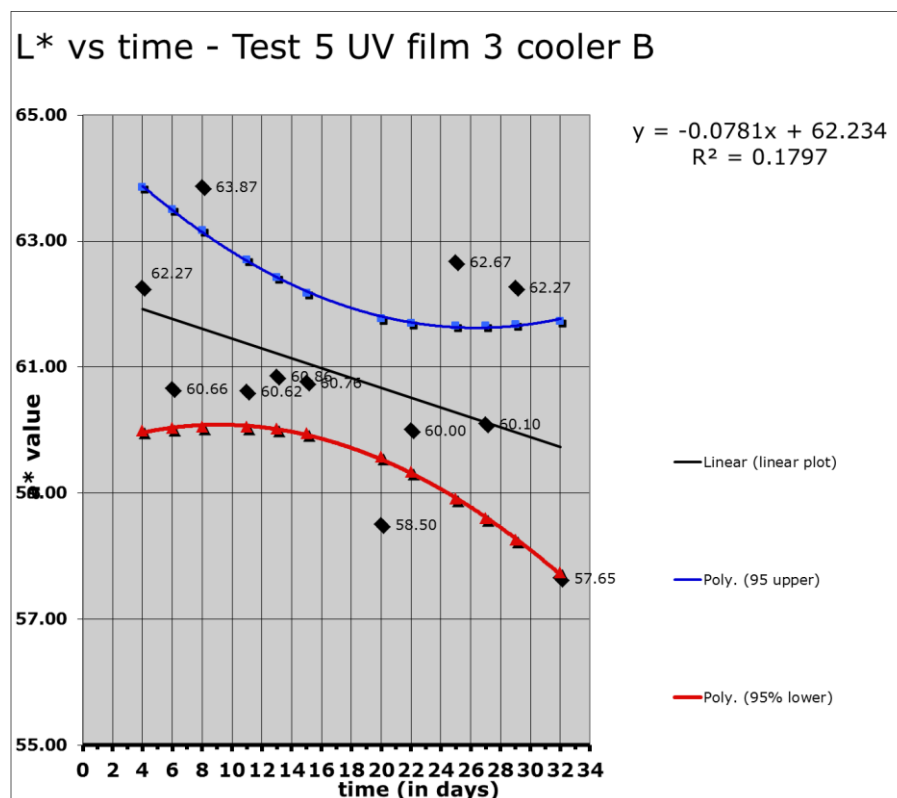
**Figure 4.61** Test 5 zero order plot of  $L^*$  vs. time (32 days) with 95 % confidence limits calculation for UV test film 1 cooler A

**Table 4.95** Test 5  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL control cooler B

1. Raw Data:		Test 5 control cooler B																	
# data pairs	Total=	12 This is automatically counted																	
Y units	L*																		
X units	days																		
STATISTICS																			
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry of (y1-yes)^2																			
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average				
61.24	4	3749.93	61.24	61.46	16.00	61.24	61.46	0.05	186.78	244.95	16.00	63.79	59.13	4.66	61.46				
64.34	6	4139.64	64.34	61.21	36.00	64.34	61.21	9.83	136.11	386.04	36.00	63.30	59.11	4.20	61.21				
60.35	8	3641.72	60.35	60.95	64.00	60.35	60.95	0.36	93.44	482.77	64.00	62.83	59.07	3.76	60.95				
60.14	11	3616.82	60.14	60.56	121.00	60.14	60.56	0.18	44.44	661.54	121.00	62.16	58.96	3.20	60.56				
59.20	13	3504.64	59.20	60.30	169.00	59.20	60.30	1.21	21.78	769.60	169.00	61.75	58.85	2.90	60.30				
58.12	15	3378.32	58.12	60.04	225.00	58.12	60.04	3.69	7.11	871.85	225.00	61.39	58.70	2.69	60.04				
57.88	20	3350.08	57.88	59.40	400.00	57.88	59.40	2.31	5.44	1157.60	400.00	60.73	58.07	2.66	59.40				
57.63	22	3320.83	57.63	59.14	484.00	57.63	59.14	2.29	18.78	1267.79	484.00	60.57	57.71	2.85	59.14				
62.36	25	3888.77	62.36	58.75	625.00	62.36	58.75	13.01	53.78	1559.00	625.00	60.41	57.10	3.31	58.75				
60.82	27	3699.48	60.82	58.50	729.00	60.82	58.50	5.42	87.11	1642.23	729.00	60.34	56.65	3.70	58.50				
57.61	29	3319.30	57.61	58.24	841.00	57.61	58.24	0.39	128.44	1670.79	841.00	60.30	56.18	4.12	58.24				
56.71	32	3215.65	56.71	57.85	1024.00	56.71	57.85	1.31	205.44	1814.61	1024.00	60.26	55.44	4.82	57.85				
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average				
slope=												-0.1290		Standard Error		2.00			
intercept=												61.9794		Sum (yi-yes)		7722.93			
rsq=												0.2914		n		12.00			
± 95% slope												0.1419		t 95%,2,n-2=		2.23			
k upper												0.0129		x average =		17.67			
k lower												-0.2710							
Equations														Sum (xi-xav		1612.89			
Y = 61.9794												-0.1290		* time		(Sum x)^2		44944.00	
																Sum(y^2)		42825.18	
																sum y		716.40	
																Sum (xi*yi)		12528.77	
																sum x		212.00	
																sum (X^2)		4734.00	



**Figure 4.62** Test 5 zero order plot of  $L^*$  vs. time (32 days) with 95 % confidence limits calculation for control cooler B

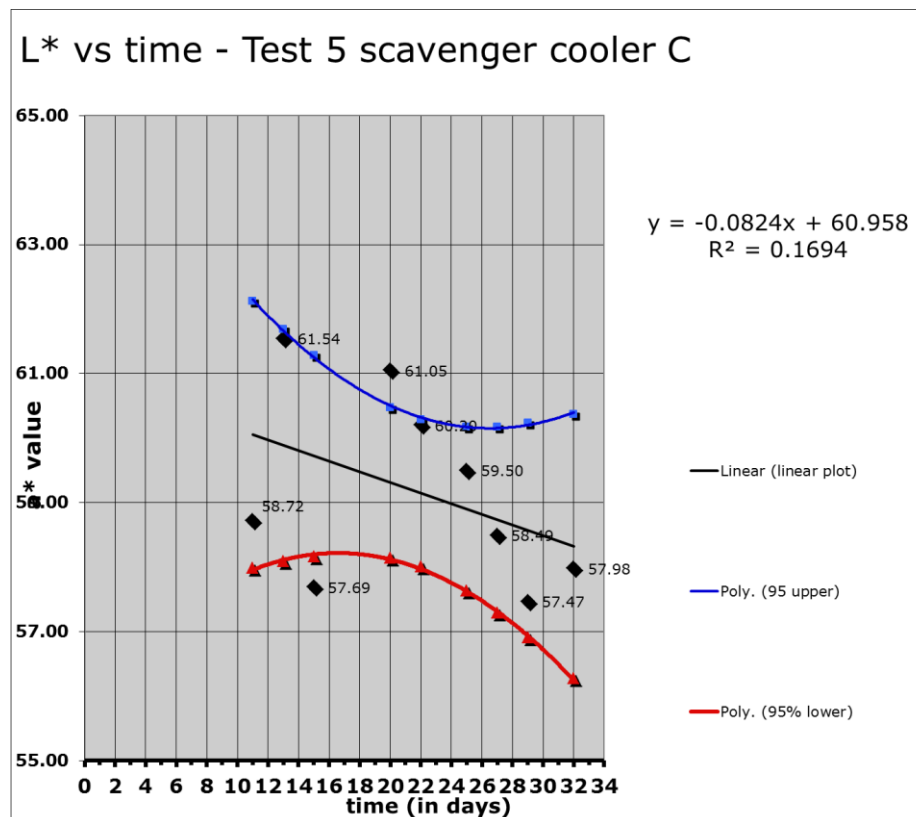
[illegible]

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**Table 4.98** Test 5  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL  $O_2$  scavenger cooler C

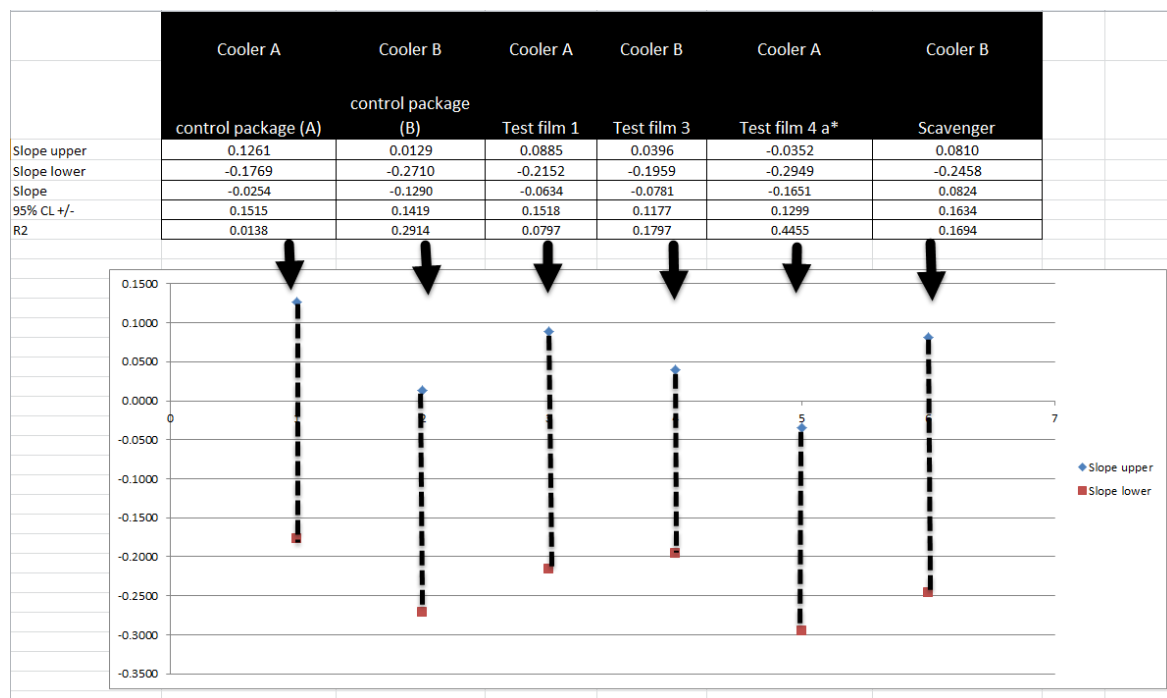
1. Raw Data:		Test 5 oxygen scavenger															
# data pairs	Total=	9	This is automatically counted														
Y units	L*																
X units	days																
STATISTICS																	
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry																	
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	
	58.72	11	3448.04	58.72	60.05	121.00	58.72	60.05	1.77	111.42	645.92	121.00	62.12	57.99	4.13	60.05	
	61.54	13	3787.17	61.54	59.89	169.00	61.54	59.89	2.73	73.20	800.02	169.00	61.69	58.08	3.61	59.89	
	57.69	15	3328.14	57.69	59.72	225.00	57.69	59.72	4.13	42.98	865.35	225.00	61.29	58.16	3.13	59.72	
	61.05	20	3727.10	61.05	59.31	400.00	61.05	59.31	3.03	2.42	1221.00	400.00	60.48	58.14	2.33	59.31	
	60.20	22	3624.04	60.20	59.15	484.00	60.20	59.15	1.11	0.20	1324.40	484.00	60.29	58.07	2.28	59.15	
	59.50	25	3540.25	59.50	58.90	625.00	59.50	58.90	0.36	11.86	1487.50	625.00	60.17	57.83	2.34	58.90	
	58.49	27	3421.08	58.49	58.73	729.00	58.49	58.73	0.06	29.84	1579.23	729.00	60.18	57.29	2.89	58.73	
	57.47	29	3302.80	57.47	58.57	841.00	57.47	58.57	1.21	55.42	1666.63	841.00	60.23	56.90	3.33	58.57	
	57.98	32	3361.68	57.98	58.32	1024.00	57.98	58.32	0.12	109.09	1855.36	1024.00	60.37	56.27	4.10	58.32	
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	
	slope=		-0.0824														
	intercept=		60.9582														
	rsq=		0.1694														
	± 95% slope		0.1634														
	k upper		0.0810														
	k lower		-0.2458														
	Equations																
	Y = 60.9582		-0.0824		* time												
	Standard Error 1.44																
	Sum (yi-yes) 18594.04																
	n 9.00																
	t 95%,2,n-2= 2.37																
	x average = 21.56																
	Sum (xi-xav) 2759.43																
	(Sum x)^2 37636.00																
	Sum (y^2) 31540.30																
	sum y 532.64																
	Sum (xi*yi) 11445.41																
	sum x 194.00																
	sum (X^2) 4618.00																



**Figure 4.65** Test 5 zero order plot of  $L^*$  vs. time (32 days) with 95 % confidence limits calculation for  $O_2$  scavenger cooler C

Applying the reaction kinetics model for food quality changes established by Labuza (in this application lightening or darkening of the product as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the slope ranges ( $+k$  for increase in  $L^*$  value (fading) over the shelf life,  $-k$  for darkening or decreasing  $L^*$  value at  $\pm$  95% CL) between treatments is provided in Table 4.99.

**Table 4.99**  $L^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in test 5 as established by Labuza' Reaction kinetics shelf life model



Predicted slopes are negative for all treatments (Figures 4.60 – 4.65). The potential for  $L^*$  being positive (lightening) over time is greatest in the control package from cooler A, UV test film 1, and the scavenger sachet package, but all overlap with the other test packages which means statistically there is not a difference between treatments.

#### 4.5.6 – Visual appearance of ham Test 5

Visual discoloration was noted in all treatments at different points throughout the study (Appendix E.1-E.7). At day 8, visually all of the treatments appear pink from samples in lanes C and D (E.3) with O<sub>2</sub> percentages ranging from 0.31 to 0.40. Comparatively, samples on day 4, 13 and 15 show significant discoloration on samples closer to the light source with similar O<sub>2</sub> percentages. These observations support greater development of discoloration near the light source. The trend toward the three lanes closest the light source (A, B, and C) developing the most distinctly discolored appearance continued in this study. Future studies should focus on lanes A-C only.

#### 4.5.7 – Cooler temperatures Test 5

The coolers utilized in this study were the same coolers used Test 4. The settings were not adjusted. Temperature was not tracked in this study. In Tests 2-4, the coolers demonstrated a consistent average temperature from 0.0° to 1.0 C° (Table 4.100).

**Table 4.100** Summary of cooler temperatures from Tests 2-4

Test 2			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.4	-3.5	3
B	0.1	-5	4
C	0.3	-4.5	5
Test 3			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.1	-5	4
B	0.6	-3	3.5
C	0.5	-4.5	4.5
Test 4			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.4	-4.5	5.5
B	1.0	-2.5	5.5

#### 4.5.8 – Conclusions Test 5

The outcome of this test was similar to test 2b, and confirms that reducing UV light exposure is ineffective for slowing meat discoloration if visible light is present.

Proximity to the light source is a key factor for the development of discoloration in a low oxygen package (>0.5) as demonstrated by the lowest *a*\* values occurring in Lanes A-C.



This test confirms that the 30 cc scavenger is not effectively removing all residual oxygen. As a result, color fading and discoloration were noted on all samples in the scavenger packaged treatment at some point during the 30 day refrigerated shelf life. Because the samples with the scavenger did not achieve 0% oxygen in all packages, there is a need to explore a scavenger with greater capacity. The success reported by other researchers with oxygen scavengers (consistently improving sliced ham color when packaged alone in a MAP package) keeps oxygen scavengers as an area of continued focus. (Anderson and Rasmussen, 1992; Chaiyapechara, Meng and Hotchkiss, 1998; Cerioli et al., 2009)

Based on observation of visual discoloration and corresponding low  $a^*$  values occurring in lanes A - C, these three lanes nearest the light source are of greatest interest moving forward. While this limits the number of samples in the study (due to limited space near the light source), the results of the testing to date has found very little discoloration in low oxygen (0.5) products that are more than 13.5" from the light source.

It is possible that not enough UV blocking or absorbing additive was added to achieve the desired effect. A potential re-test could be to add a greater percentage of the UV additives to the sealant layer, but with the potential of increasing the haze / distorting the visual acceptance of the product by the consumer, added cost to the product, and evidence that light in the visible spectrum is equally as harmful, additional tests of UV film alone are not recommended. Another potential test for using a UV reduction film could be to find alternatives that reduce UV exposure 100% at key wavelengths of interest. While the UV Pet blocked 87% at 360 nm and 16% at 400 nm, and UV sealant #1 blocked 90% at 375 nm and 60% at 400 nm; neither blocked 100% at 366 nm and 400 nm which are wavelengths identified to cause photooxidation damage.

## **4.6 - Test 6 Proximity of sandwich to the light source**

### **4.6.1 – Test 6 overview**

The purpose of this test was to determine if there was a significant difference in discoloration development based on proximity to the light source by comparing the performance in the first three adjacent lanes. The position nearest the light source is referred to as “lane A” (which is approximately 0.5” to 4.5” from the fluorescent lighting based on the width of the sandwich), the second position “lane B” (4.6” to 9.1” from the fluorescent lighting), and the third position “lane C” (9.2” – 13.7” from the fluorescent lighting).

In the previous Tests 1-5, it has been observed that during refrigerated shelf life, the minimum  $a^*$  values have occurred in lanes A – C only. Literature regarding the impact of light intensity on meat color is inconsistent. Many of the studies agree that discoloration is proportional to light level and exposure time (Sylvania). However, in a study of sliced cooked cured ham in vacuum packaging, Li et al. found that illumination had no significant effect on the  $a^*$  value across the conditions of 1000, 200, and 0 lux through 28 days storage, but it was observed that at the condition of 0 lux at day 35, there was statistically higher  $a^*$  values compared to the other light intensity treatments. Color was measured with a Minolta chromameter CR-400 (Li et al., 2012).

It has been an E.A. Sween company sales strategy to place ham sandwiches away from the light source to help prevent discoloration, but it has not been validated that this is effective, and if it is, the proper distance has not been established.

### **4.6.2 – Methods and Materials Test 6**

This study marked a change in the cooler set method and the assembly and packaging of the product. To increase the surface area of ham exposed to the light, the sandwiches were assembled in the order of (bottom to top) bread, bread, cheese, and ham with the ham now being placed flat on the top surface for full light exposure (Figure 4.66 – right

side). The sandwich assembly was previously bread, ham, cheese, bread and was sliced on the bias to expose the middle of the sandwich for the appearance of a wedge shape with “bunched” meat (Figure 4.66 – left side).

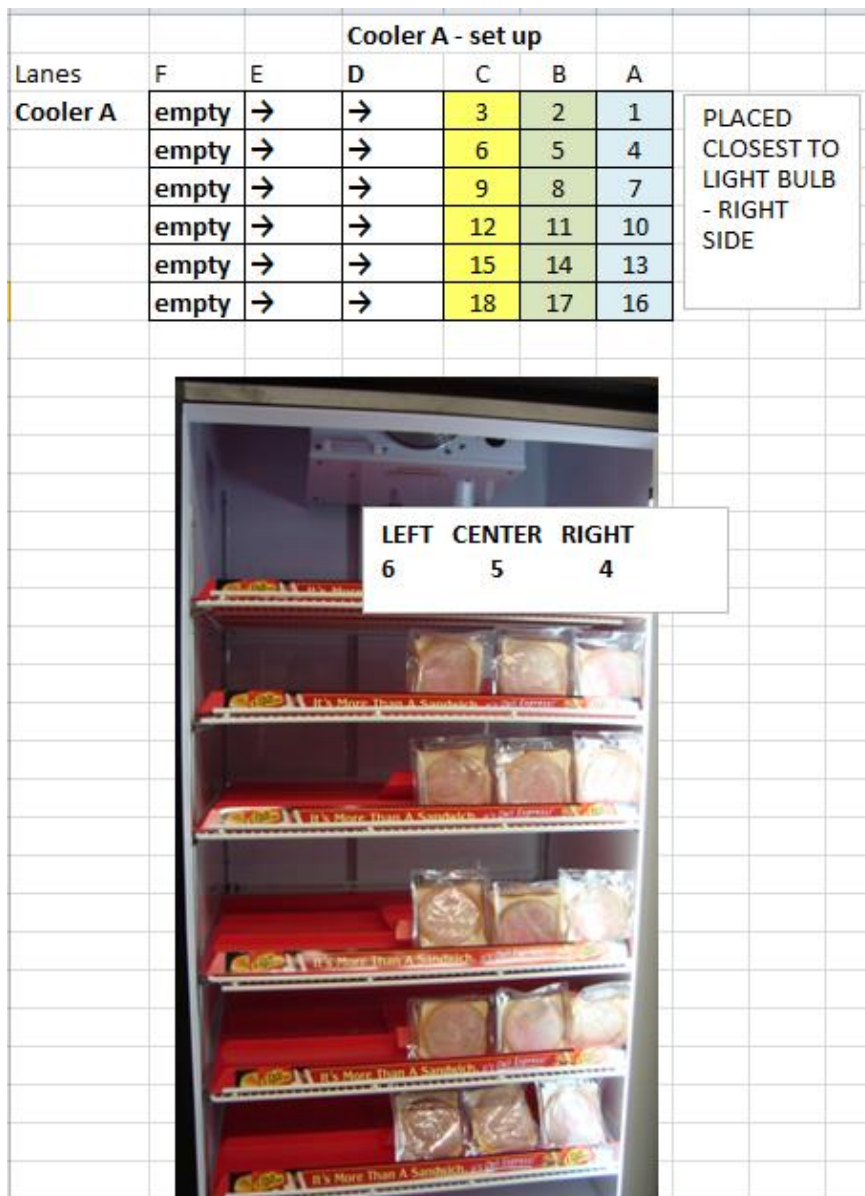


**Figure 4.66** Prior packaging format (wedge shape) compared to new format (flat face ham). The change was made to help maximize the surface area exposed to light for better  $L^*$  and  $a^*$  color analysis given the available lens diameter for the chromameter. With this method, 100% of the surface measured is exposed to light compared to the previously estimated 24%

The sandwiches use the same ham, cheese and bread formulations and weights used in previous tests, but the ham was not “bunched” and the sandwich was not sliced into the wedge format the consumer sees. The new package configuration (flat face sandwiches) has a product to package ratio of 1 to 1.8 (Section 3.14) compared to the 1 to 1 ratio of the wedge format. This ratio difference creates the potential for greater oxygen levels by increased void space in the package. In a study of sliced cured ham to optimize color stability during packaging and retail display, Møller et al. found that the interaction between measured oxygen percentage in the head space and the product to headspace volume ratio was critical. A low head space oxygen level wasn’t enough if the headspace volume is large and thus sufficient oxygen will be available for discoloration to take place (Møller et al., 2002). The Møller et al. study compared a head space ratio of 1:1.3

to 1: 4.9. Both had a measured oxygen content of 0.1%. The outcome was lower  $a^*$  scores for the larger ratio package (Møller et al., 2002).

A Beverage Air cooler (Model # LV27 c) with fluorescent lighting was used in this study. Based on the observations from the previous tests that visual discoloration is more dominate in the first three lanes from the light source, product was placed only in the first three vertical lanes to maximize exposure to light (Figure 4.67).



**Figure 4.67** Cooler set up and sandwich placement for Test 6

A sandwich from each lane was evaluated six times throughout the 32 day refrigerated shelf life test by testing for oxygen in the headspace,  $L^*$  and  $a^*$  color analysis, and visual inspection (documented with a photograph (Appendix F.1-F.6)) using the methods outlined in tests 2-5. The sampling dates were selected with a goal of evaluating approximately every 7 days (Table 4.101).

**Table 4.101** Sampling dates and sandwich numbers evaluated during Test 6

Day	Date	Sandwich number evaluated Lane A	Sandwich number evaluated Lane B	Sandwich number evaluated Lane C
<b>Sandwich produced on:</b>	<b>12/5/2012</b>			
Day 0	1/10/2013			
Day 1	1/11/2013	1	2	3
Day 6	1/16/2013	4	5	6
Day 14	1/24/2013	7	8	9
Day 21	1/31/2013	10	11	12
Day 26	2/5/2013	13	14	15
Day 32	2/11/2013	16	17	18

All sandwiches were assembled and placed in MAP (Modified Atmosphere Packaging) with an 80% N<sub>2</sub> / 20% CO<sub>2</sub> blend (Materials and Methods section 3.15) at E.A. Sween Company using a Multivac R530 (Materials and Methods section 3.19).

The clear 8 millimeter bottom forming film used was CURLON<sup>®</sup> (Grade 9581-AA) manufactured by Curwood<sup>®</sup> in Osh Kosh, WI (section 3.4 Methods and Materials).

After forming, the minimum pouch thickness is 1 millimeter. The clear top non-forming film used is a lamination of a 50 gauge polyethylene terephthalate (PET) / 200 gauge peelable linear low density polyethylene (LLDPE) co-extrusion (two ply lamination) that is 2.6 millimeter thick, produced by Belmark in De Pere, WI (section 3.3 Methods and Materials). Sandwiches were not labeled to maximize light exposure.

The ham, cheese and bread utilized are the same formulations used in tests 1-5. All materials for each lane are pulled from the same production lot codes to minimize material variability. The ham and cheese was stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly.

The bread was stored at room temperature (approximately 21° C) prior to assembly. The length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The sandwiches spent 36 days in dark frozen storage before evaluation for refrigerated shelf life.

To establish the strength of the fluorescent light reaching the sandwich, an ExeTech Light meter model 401025 (Nashua, NH) was used. The most sensitive setting of 1999/2000 was selected. X10 setting was used for indoor measurements. The challenge in establishing the strength of light reaching the ham surface is the variability in how the product sits on the shelf. Unlike a can with a rigid, stable structure, the sandwich packing is pliable and often can become misshapen in the shipping container, resulting in high variability in how the sandwich sits on the shelf. If the sandwich is lying flat on the shelf compared to fully upright, the intensity reaching the surface can vary as much as 1100 lux in lane A (Table 4.102). The difference in intensity decreases the further the sandwiches are removed from the light source and with a decreasing angle on the shelf. The intensity of light source measurement unit is Lumen (Lumen). Intensity of illumination of the measuring unit is Lux. The relationship between the two is 1 Lux = 1 Lumen/m<sup>2</sup> (NO.1 lighting technology Ltd., 2015).

**Table 4.102** Strength of light reaching the product (in lux). To convert lux to watt, multiply lux value by 0.0079. 1550 lux was the highest reading on the sandwich surface (12.2 watt of a 32 watt bulb). 2550 lux is the output of the fluorescent light as reported by the bulb manufacturer (Section 3.18 Methods and Materials)

Position	Lane C (lux) approx. 9.2” to 13.7” from light source	Lane B (lux) approx. 4.6” to 9.1” from light source	Lane A (lux) approx. 0.5” to 4.5” from light source	(2550 lux) light source
Flat	207	290	450	
Angled	392	763	1550	

### 4.6.3 – Oxygen (O<sub>2</sub>) percentages for packages in Test 6

The results of the oxygen percentages in the head space over time are reported in Table 4.103.

**Table 4.103** Oxygen percentages by package Test 6

Day	lane A	lane B	lane C
1			
6	1.56	0.10	0.06
14	0.00	0.00	0.00
21	0.00	0.00	0.00
26	0.00	0.00	0.00
32	0.00	0.00	0.00
min	0.00	0.00	0.00
max	1.56	0.10	0.06
range	1.56	0.10	0.06

Day 1 oxygen readings were not obtained because of lack of availability of the Mocon equipment (Section 3.22 Materials and Methods). At day 6, the O<sub>2</sub> values in lanes B and C were consistent, but the 1.56 % reading in lane A is higher than would be expected. (Given data that initial O<sub>2</sub> levels can increase to 1.5% post packaging, but would drop within the first 48 hours of frozen storage). By day 14, all packages proceed to zero percent residual oxygen in the head space. (Table 4.103) Given the amount of visual discoloration on the ham (Appendix F.1-F.6), the conclusion is oxygen was consumed by converting nitrosylhemochrome to metmyoglobin. This is more than seen in previous tests due to the increased area of exposure and the larger product to headspace ratio. In the previous test 5, the range of oxygen percentages in the headspace at day 6 was 0.31 to 0.42% in the wedge format. Sandwiches in the wedge format study did not reach 0% O<sub>2</sub> in the headspace during the study.

#### 4.6.4 – $a^*$ scores Test 6

The variability of  $a^*$  values within and across all lanes over time was substantial with a range of scores from 3.91 to 19.35 (Table 4.104). The raw data for  $L^*$  and  $a^*$  values is in Appendix F.7.

**Table 4.104** –  $a^*$  value scores for lanes A, B, and C in Test 6.

Day	lane A	lane B	lane C
1	14.56	16.14	16.55
6	15.78	12.52	19.35
14	12.83	17.18	14.87
21	11.36	16.25	12.10
26	3.91	15.74	11.71
32	17.24	11.13	13.85
min	3.91	11.13	11.71
max	17.24	17.18	19.35
range	13.33	6.05	7.64

The larger range over time for lane A sandwiches is due to a low  $a^*$  score towards the end of the refrigerated shelf life. The minimum  $a^*$  values achieved in lane A occurred on day 26 (3.91) with an oxygen percentage of 0. The score of  $a^* = 3.91$  is the lowest recorded value in all tests 1-6. Lane B had a minimum  $a^*$  value occur at day 32 (11.13), and lane C had its lowest recorded  $a^*$  value at day 26 (11.71). There is no evidence that the lane A sandwich at day 26 was a leaker or was abused. This leads to the conclusion that the lower score is the result of greater metmyoglobin formation given the location in the cooler, initial oxygen content, product to package ratio, and greater exposed surface area of ham.

Compared to previous tests in the wedge format, the range of  $a^*$  values using the flat faced ham package method are similar in lanes B and C (Table 4.105). In the previous tests, the minimum  $a^*$  has been observed as early as day 7, and as late as day 25. In this study, all minimums occurred after day 25.



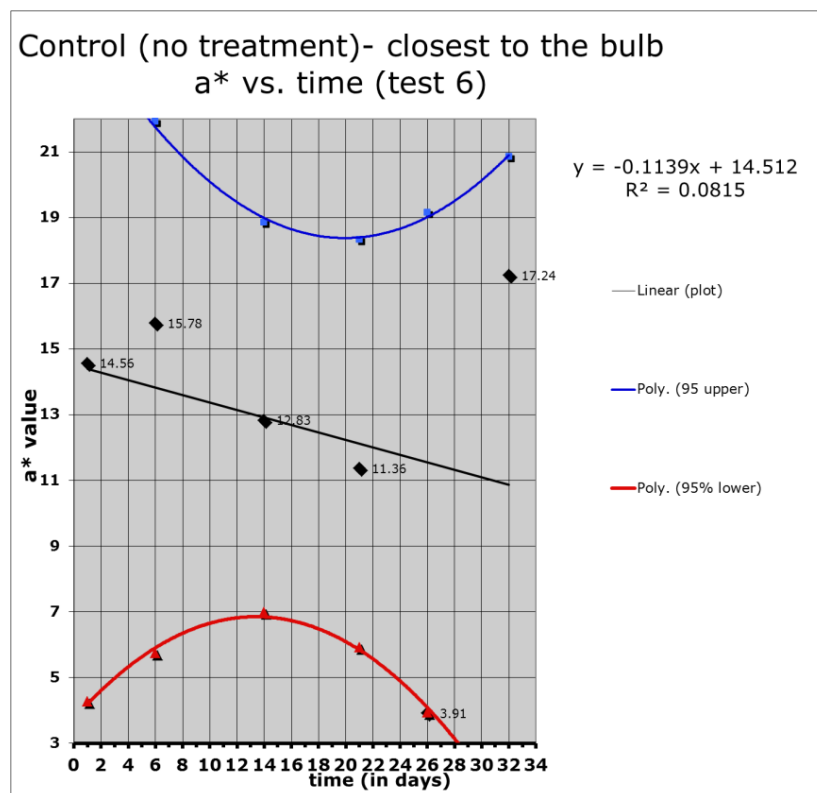
**Table 4.105**  $a^*$  minimum, maximum and ranges for Tests 2 – 5 for the control packages in the wedge shaped format

	TEST 2	TEST 3	TEST 4	TEST 5
	Control cooler A	Control cooler A	Control cooler A	Control Cooler A
	$a^*$	$a^*$	$a^*$	$a^*$
	14.35	16.89	16.42	18.34
	15.46	17.41	16.19	18.09
	13.48	19.42	12.18	15.83
	13.54	17.55	18.85	17.11
	10.45	17.90	18.75	14.77
	14.52	17.91	18.23	16.05
	13.97	18.94	16.69	18.28
	12.75	17.22	17.33	18.13
	11.41	16.16	18.29	15.81
	14.93	19.73	19.53	17.82
	17.59	17.72	18.22	17.95
	18.38	18.53		14.86
	11.18	18.69		
	11.94	18.69		
	13.35			
	10.43			
	18.89			
	18.66			
	17.88			
	10.48			
	14.31			
	14.22			
min	10.43	16.16	12.18	14.77
max	18.89	19.73	19.53	18.34
range	8.45	3.57	7.35	3.57

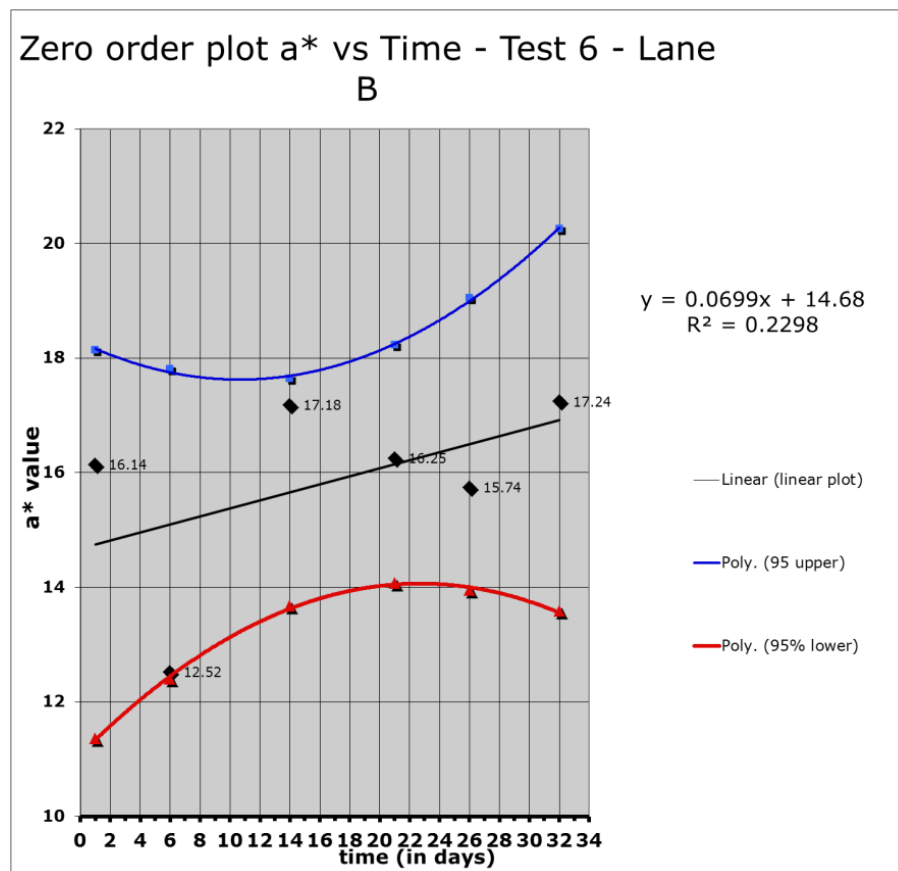
Entering the  $a^*$  values from Table 4.104 above into the kinetics data input sheet (Tables 4.106 – 4.108) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

**Table 4.106** Test 6  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for sandwiches in Lane A

1. Raw Data:																
# data pairs Total=			6 This is automatically counted													
Y units			a* Lane A													
X units			days													
2. Calculati																
Note after entering Y and X you need to pull down formulas in each column from top to last entry rd(yi-yes)^2																
STATISTICS																
			(x1-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte							
									average							



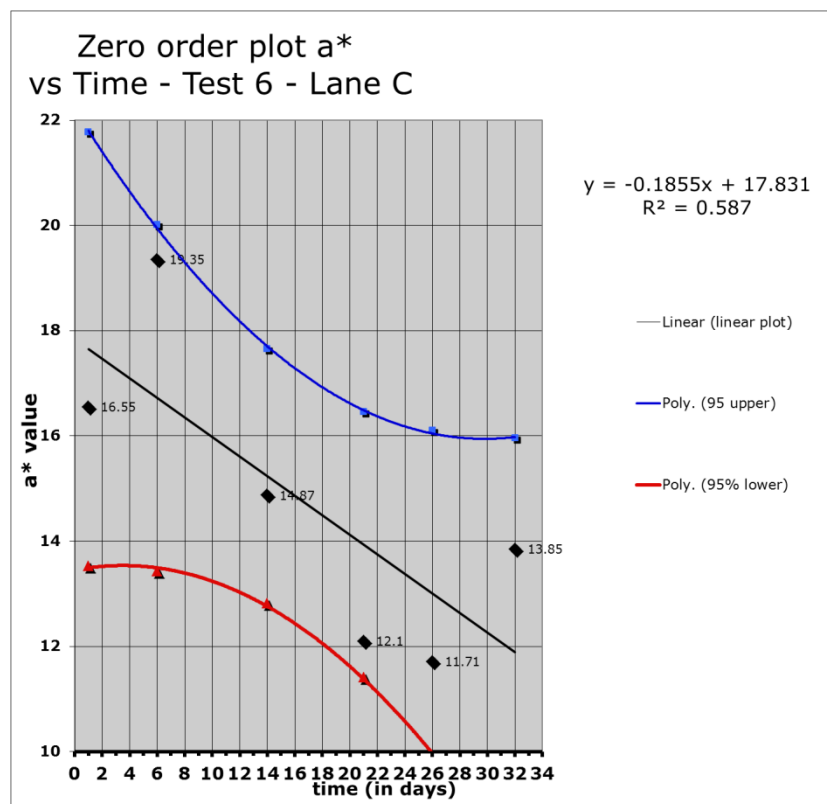
**Figure 4.68 A:** Control Ham lane A (nearest the bulb) Zero order plot of  $a^*$  vs. time test 6 (26 days) with 95 % confidence limits calculation.

[illegible]

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**Table 4.108** Test 6  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for Lane C sandwiches.

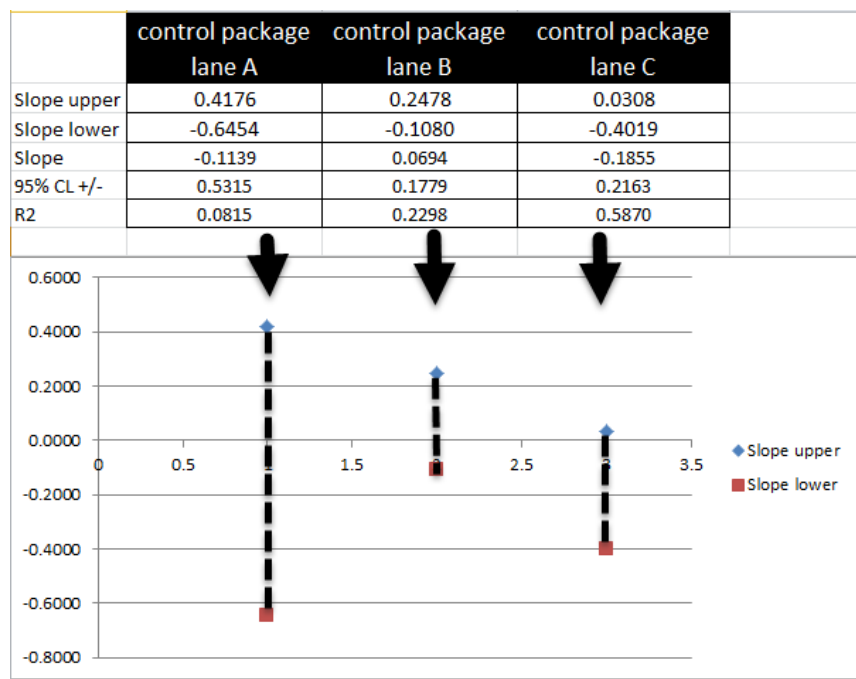
1. Raw Data:																
# data pairs Total=		6 This is automatically counted														
Y units		a*														
X units		days														
STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yes)^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	16.55	1.0	273.90	16.55	17.65	1.00	16.55	17.65	1.20	245.44	16.55	1.00	21.77	13.52	8.25	17.65
	19.35	6.0	374.42	19.35	16.72	36.00	19.35	16.72	6.93	113.78	116.10	36.00	20.01	13.42	6.59	16.72
	14.87	14.0	221.12	14.87	15.23	196.00	14.87	15.23	0.13	7.11	208.18	196.00	17.65	12.81	4.84	15.23
	12.1	21.0	146.41	12.10	13.93	441.00	12.10	13.93	3.36	18.78	254.10	441.00	16.46	11.41	5.06	13.93
	11.71	26.0	137.12	11.71	13.01	676.00	11.71	13.01	1.68	87.11	304.46	676.00	16.10	9.91	6.20	13.01
	13.85	32.0	191.82	13.85	11.89	1024.00	13.85	11.89	3.83	235.11	443.20	1024.00	15.96	7.83	8.13	11.89
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	slope=													Standard Error	2.07	
	intercept=													Sum (yi-yes)	1606.82	
	rsq=													n	6.00	
	± 95% slope													t 95%, 2, n-2=	2.78	
	k upper													x average =	16.67	
	k lower													Sum (xi-xav	2096.22	
	Equations															
	Y = 17.8308 -0.1855 * time															
														(Sum x)^2	10000.00	
														Sum(y^2)	1344.80	
														sum y	88.43	
														Sum (xi*yi)	1342.59	
														sum x	100.00	
														sum (X^2)	2374.00	



**Figure 4.70 A:** Control Ham lane C Zero order plot of  $a^*$  vs. time (26 days) test 6 with 95 % confidence limits calculation.

Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the slope ranges ( $+k$  for increase in  $a^*$  value (redness) over the shelf life,  $-k$  for loss of redness or decreasing  $a^*$  value at  $\pm$  95% CL) between treatments is provided in Table 4.109.

**Table 4.109**  $a^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in Test 5 as established by Labuza's Reaction kinetics shelf life model



Lane A demonstrates the greatest range of slopes both positive and negative over time. Lane B demonstrates the smallest range of potential outcomes with a greater likelihood of a positive slope over time. Lane C demonstrates a greater potential for a negative slope. Part of the Lane A large range variability is a result of the day 26 outcome of  $a^* = 3.91$ . Because this sample demonstrated that it maintained the MAP environment over time ( $O_2\% = 0\%$ ), the result shouldn't be removed and is attributed to the changes in packaging method and amount of ham exposed.

The trend line slope of the line for both lane A and C was decreasing (loss of redness), while the second lane was increasing (improving redness) over time (Figures 4.65 – 4.67). The  $R^2$  values for lane C reflects a good fit for the data, while lanes A & B have a poor fit of data (Figures 4.65 – 4.67 above). The fit of the data is both a reflection of a small sample size and the high variability between scores over time.

#### 4.6.5 – $L^*$ scores Test 6

The variability of  $L^*$  values within and across all lanes over time was also substantial with a range of scores from 57.36 to 65.47 (Table 4.110). The range, minimum and maximum  $L^*$  values is similar for all lanes, with lane A having the largest range (Table 4.110). The differences in ranges between lanes on  $L^*$  were not as large as observed for  $a^*$  value. As observed in other testing,  $L^*$  and  $a^*$  outcomes don't always correlate.

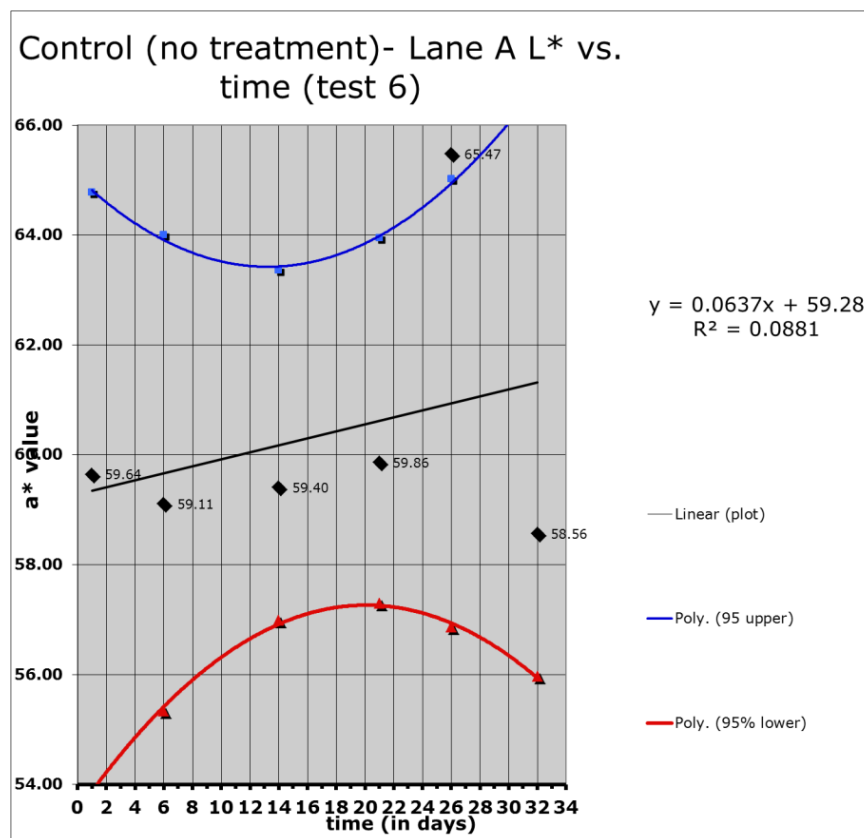
**Table 4.110**  $L^*$  values for lanes A, B, and C in Test 6

Day	lane A	lane B	lane C
1	59.64	64.63	63.19
6	59.11	63.84	59.26
14	59.40	59.01	59.14
21	59.86	59.08	62.04
26	65.47	58.60	61.37
32	58.56	58.98	57.36
min	58.56	58.60	57.36
max	65.47	64.63	63.19
range	6.91	6.03	5.84

Entering the  $L^*$  values from Table 4.110 above into the kinetics data input sheet (Tables 4.111 – 4.113) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of pigment reactions causing the meat to become grey or the concentration of pigments which is the result of moisture loss.

**Table 4.111** Test 6  $L^*$  data input sheet for establishing slope, slope upper and lower value at the 95% CL for Lane A sandwiches.

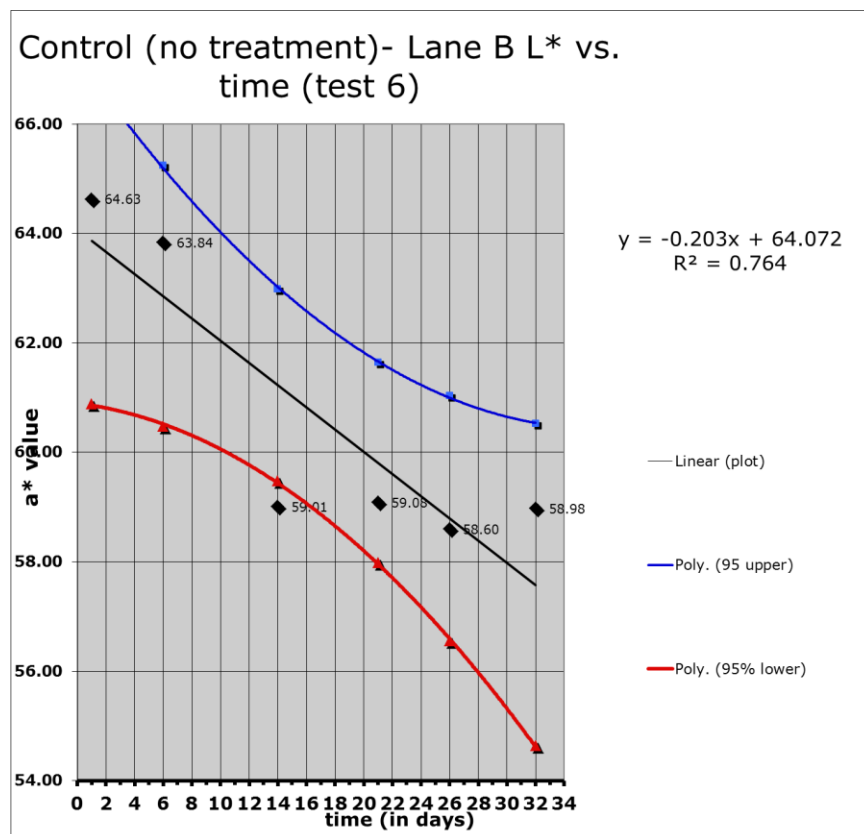
1. Raw Data:															
# data pairs	Total=	6	This is automatically counted												
Y units	L*	Lane A													
X units	days														
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry (d/yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte								
average															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte
59.64	1.0	3557.33	59.64	59.34	1.00	59.64	59.34	0.09	245.44	59.64	1.00	64.77	53.91	10.86	59.34
59.11	6.0	3493.99	59.11	59.66	36.00	59.11	59.66	0.30	113.78	354.66	36.00	64.00	55.33	8.67	59.66
59.40	14.0	3528.76	59.40	60.17	196.00	59.40	60.17	0.59	7.11	831.65	196.00	63.36	56.99	6.37	60.17
59.86	21.0	3583.62	59.86	60.62	441.00	59.86	60.62	0.57	18.78	1257.13	441.00	63.95	57.29	6.66	60.62
65.47	26.0	4286.32	65.47	60.94	676.00	65.47	60.94	20.56	87.11	1702.22	676.00	65.02	56.86	8.16	60.94
58.56	32.0	3429.27	58.56	61.32	1024.00	58.56	61.32	7.61	235.11	1873.92	1024.00	66.67	55.97	10.71	61.32
		0.00	0.00	59.28	0.00	0.00	59.28	3514.13	277.78	0.00	0.00	64.95	53.61	11.33	59.28
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte
															average
slope=		0.0637													
intercept=		59.2801													
rsq=		0.0881													
± 95% slope		0.2849													
k upper		0.3486													
k lower		-0.2212													
Equations															
Y = 59.2801		0.0637		* time											



**Figure 4.71 A:** Control Ham lane A Zero order plot of  $L^*$  vs. time (26 days) test 6 with 95 % confidence limits calculation.

**Table 4.112** Test 6  $L^*$  data input sheet for establishing slope, slope upper and lower value at the 95% CL for Lane B sandwiches.

1. Raw Data:															
# data pairs		Total=	6 This is automatically counted												
Y units	L*		Lane B												
X units	days														
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry (d <sub>yi-yes</sub> )^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte	average							
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte
64.63	1.0	4176.61	64.63	63.87	1.00	64.63	63.87	0.57	245.44	64.63	1.00	66.86	60.88	5.98	63.87
63.84	6.0	4075.55	63.84	62.85	36.00	63.84	62.85	0.97	113.78	383.04	36.00	65.24	60.47	4.77	62.85
59.01	14.0	3482.18	59.01	61.23	196.00	59.01	61.23	4.93	7.11	826.14	196.00	62.98	59.48	3.51	61.23
59.08	21.0	3490.84	59.08	59.81	441.00	59.08	59.81	0.53	18.78	1240.75	441.00	61.64	57.98	3.67	59.81
58.60	26.0	3433.57	58.60	58.79	676.00	58.60	58.79	0.04	87.11	1523.51	676.00	61.04	56.55	4.49	58.79
58.98	32.0	3478.25	58.98	57.58	1024.00	58.98	57.58	1.96	235.11	1887.25	1024.00	60.52	54.63	5.89	57.58
		0.00	0.00	64.07	0.00	0.00	64.07	4105.18	277.78	0.00	0.00	67.19	60.95	6.24	64.07
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte

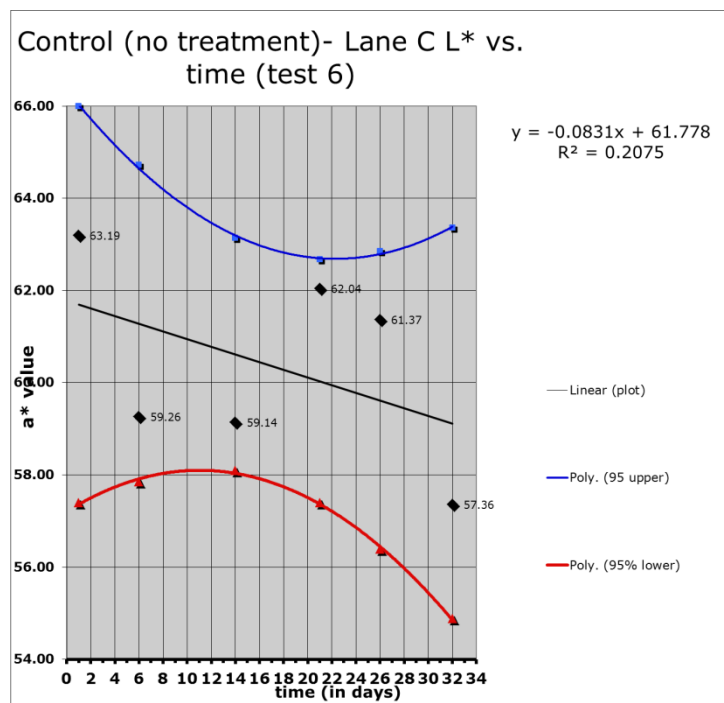


**Figure 4.72 A:** Control Ham lane B Zero order plot of  $L^*$  vs. time (26 days) test 6 with 95 % confidence limits calculation.



**Table 4.113** Test 6  $L^*$  data input sheet for establishing slope, slope upper and lower value at the 95% CL for Lane C sandwiches.

1. Raw Data:															
# data pairs Total=		6 This is automatically counted													
Y units	L*	Lane C													
X units	days														
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte	average							
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte
63.19	1.0	3993.40	63.19	61.70	1.00	63.19	61.70	2.24	245.44	63.19	1.00	66.00	57.39	8.61	61.70
59.26	6.0	3511.75	59.26	61.28	36.00	59.26	61.28	4.08	113.78	355.56	36.00	64.72	57.84	6.87	61.28
59.14	14.0	3497.93	59.14	60.61	196.00	59.14	60.61	2.16	7.11	828.01	196.00	63.14	58.09	5.05	60.61
62.04	21.0	3848.55	62.04	60.03	441.00	62.04	60.03	4.02	18.78	1302.77	441.00	62.67	57.39	5.28	60.03
61.37	26.0	3765.87	61.37	59.62	676.00	61.37	59.62	3.06	87.11	1595.53	676.00	62.85	56.38	6.47	59.62
57.36	32.0	3289.79	57.36	59.12	1024.00	57.36	59.12	3.10	235.11	1835.41	1024.00	63.36	54.88	8.49	59.12
		0.00	0.00	61.78	0.00	0.00	61.78	3816.56	277.78	0.00	0.00	66.27	57.29	8.98	61.78
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte

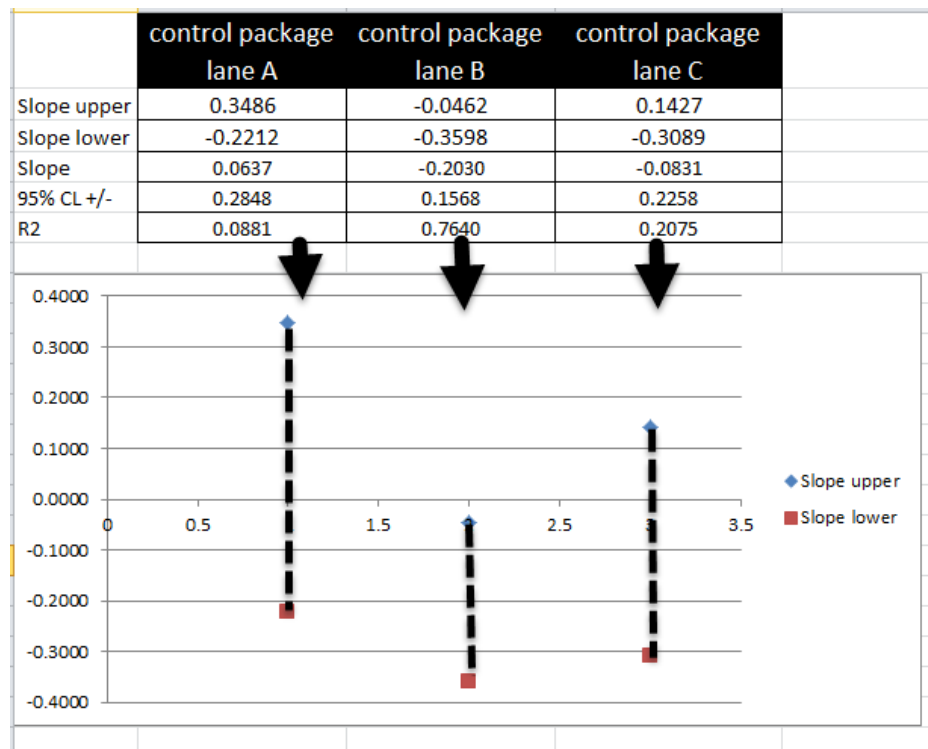


**Figure 4.73 A:** Control Ham lane C Zero order plot of  $L^*$  vs. time (26 days) test 6 with 95 % confidence limits calculation.

Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over

time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the slope ranges (+k for lightening over the shelf life, - k for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.114.

**Table 4.114**  $L^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in test 6 as established by Labuza' Reaction kinetics shelf life model.

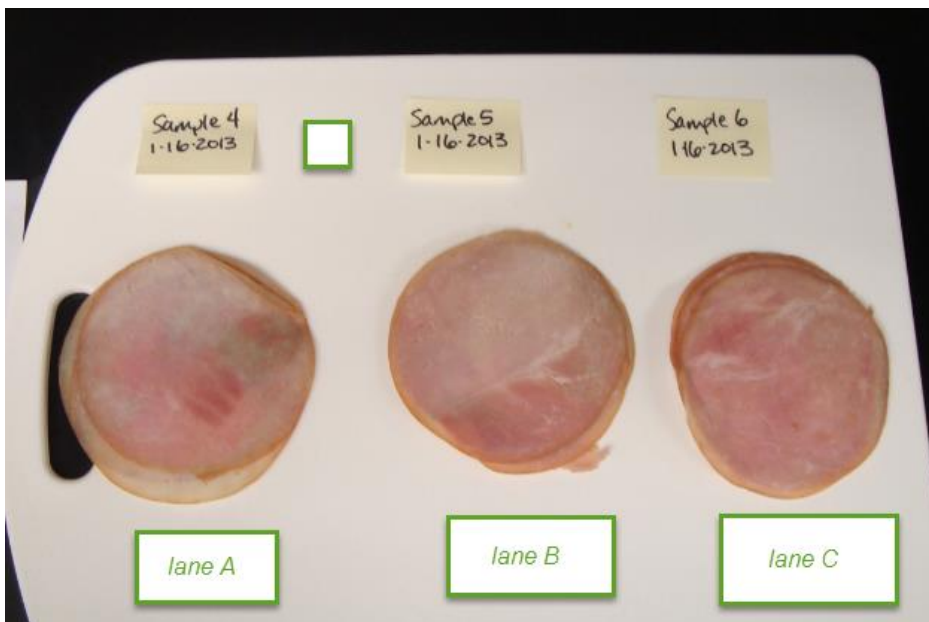


Lane A demonstrates the greatest potential for fade over time (positive slope) followed by lane C. Lane B demonstrates a negative slope as a function of time. However, there is overlap of potential outcomes for all lanes which makes the performance in each lane not statistically different. Another viewpoint of this result is that lane A has greater volatility in outcomes, with a higher risk of a negative result (in the case of  $L^*$  scores, a positive slope or lightening over time). The same statement is true for the  $a^*$  results from this test. The high variability in starting  $a^*$  and  $L^*$  values makes it more difficult to

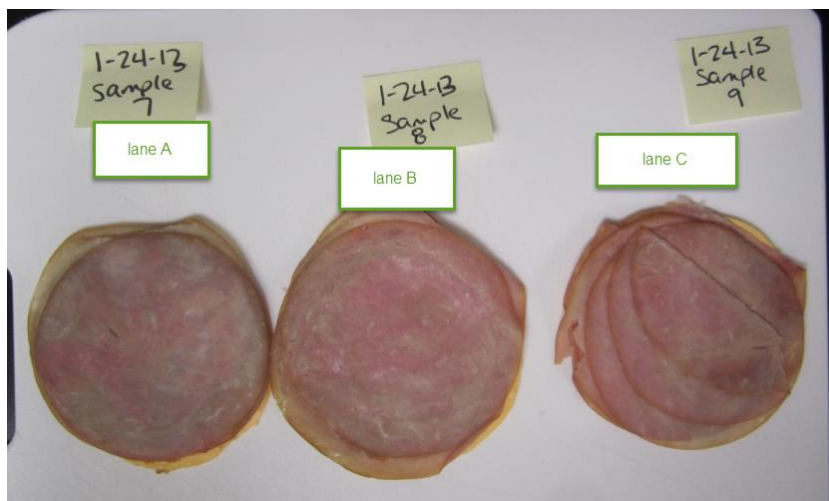
attribute the results to photooxidation, but this method of evaluation allows for the conclusion that sandwiches closest to the light source have more unpredictable outcomes compared to lane B. Lane C also demonstrates less predictable outcomes, which supports the finding that there is no statistical difference in performance based on distance from the light.

#### 4.6.6 – Visual appearance of the ham Test 6

Visual discoloration is more readily discerned in all lanes over the previous Tests 1-5. In week one, lanes A and B packages quickly developed discoloration (Figure 4.74). By week 2, all three lanes showed discoloration (Figure 4.75). Visual appearance of all days is documented in Appendix F.1 – F.6.



**Figure 4.74** Day 6 visual appearance of sandwiches in Test 6 (cooler position test)



**Figure 4.75** Day 14 appearance of ham from sandwiches in Test 6 (Cooler position test)

Visual inspection demonstrates that the product nearest the light source (lane A) has more obvious signs of metmyoglobin formation, and color appears to improve the further the product is from the light source. However the  $a^*$  and  $L^*$  kinetics slopes over time rank the color scores in order of lowest (loss of red, lighter) to highest (gain of red, darker) as 1) Lane A, 2) Lane C, 3) Lane B. (Table 4.109 and Table 4.114)

#### **4.6.7 – Cooler temperatures Test 6**

The coolers utilized were the same as test 4 (Cooler A). The settings were not changed. Refrigerated temperature was not tracked in this study. In Tests 2-4, the coolers demonstrated a consistent average temperature from 0.0° to 1.0 C° (Table 4.115).

**Table 4.115** Summary of cooler temperatures from Tests 2-4

Test 2			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.4	-3.5	3
B	0.1	-5	4
C	0.3	-4.5	5
Test 3			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.1	-5	4
B	0.6	-3	3.5
C	0.5	-4.5	4.5
Test 4			
Cooler	Average (C°)	Min (C°)	Max (C°)
A	0.4	-4.5	5.5
B	1.0	-2.5	5.5

#### 4.6.8 – Conclusions Test 6

Though greater visual discoloration is demonstrated in lane A with a greater potential of lower  $a^*$  values over time, statistically there is no difference in the rate constants for lanes A, B and C. Visual discoloration is more apparent nearest the light source as lane A was the first to develop visual discoloration and is visually more discolored compared to lanes B and C over time (Appendix F.4 – F.7). On four of six days, actual  $a^*$  values were lowest in lane A (Table 4.116), but were not statistically different given the overlap of range of predicted values for each lane over time.

**Table 4.116**  $a^*$  values over time Test 6. Lane A had the lowest  $a^*$  on 4 of 6 days of the study (highlighted in yellow)

Day	lane A	lane B	lane C
1	14.56	16.14	16.55
6	15.78	12.52	19.35
14	12.83	17.18	14.87
21	11.36	16.25	12.10
26	3.91	15.74	11.71
32	17.24	11.13	13.85
min	3.91	11.13	11.71
max	17.24	17.18	19.35
range	13.33	6.05	7.64

Standardizing the level of lumination that reaches the ham surface for testing is difficult as several factors impact the outcome including: 1) The glossy surface of the film (will reflect light); 2) the angle of the package on the store shelf (Will impact the intensity of the light reaching the surface. In this study, a difference of 70% is measured in lux strength if the sandwich facing (front of the package) is perpendicular to the refrigeration shelving compared to lying flat on the shelf), 3) Sandwiches in lanes B and beyond may be shielded by sandwiches next to them. 4) With indoor lighting providing 200-800 lux of light (No. 1 lighting, 2014), the light bulb isn't the only light source hitting the ham. Even if a test could be devised where the lumination level was consistent, and a standard distance and angle could be established, it would be impossible to execute in a retail setting. At best, a best practices could be established for distance. The results of this test support that there is no advantage to placing a sandwich 4" from the light source compared to 13.7" from the light source.

This result of no statistical difference between lanes despite measured intensity differences per lane is consistent with Li et al. finding that under Illumination of 1000, 200, and 0 lux, vacuum packed ham did not show a significant difference on  $a^*$  value other than on day 35 with the 0 lux treatment having a higher  $a^*$  value (Li et al., 2012). The greater exposure of ham surface area allowed for easier measurement of discolored areas and better visual interpretation.

A future study would be to compare the statistical performance of lanes A-C to D-F.

## 4.7 Test 7 Increased capacity oxygen scavenger and Ultraviolet (UV) film revisited

### 4.7.1 Test 7 overview

The purpose of this test was to evaluate the performance of: 1) an oxygen scavenger sachet with greater absorbing capacity (Multisorb D-50 cc – Appendix G.13); 2) the combination of the D-50 cc oxygen scavenger sachet with a UV barrier film (Belmark<sup>®</sup> UV PET/adhesive /UV PET/ adhesive / UV sealant #2 – test film #4); and 3) UV barrier test film 4. This would be done for  $a^*$  and  $L^*$  color scores, residual oxygen and development of visual discoloration over time. Previous tests 4 and 5 with Multisorb D-30 cc resulted in packages with residual oxygen remaining during the refrigerated shelf life. It is speculated that this could be attributed to four potential factors (or combinations thereof) including 1) Not enough air flow around the sachet, 2) Greater than 30cc needed to be removed (which is possible if more significant trapped air was present or if the Multivac did not remove sufficient quantities of O<sub>2</sub> up front, but neither are likely based on previous testing), 3) Not enough time prior to freezing for the sachet to remove O<sub>2</sub> (as the O<sub>2</sub> scavenging reaction becomes extremely slow at freezing temperatures) and 4) The presence of Carbon Dioxide inhibiting oxygen absorption. Carbon dioxide in the moist environment of an iron-based oxygen absorber will condense and can form ferrous carbonate as some of the iron oxidizes. It is believed that this forms on the surface of an iron particle resulting in a barrier that inhibits further oxidation (T. Powers, personal communication, February 6, 2015). While a greater capacity sachet does not address air flow or carbon dioxide inhibition, it would address capacity and the rate of O<sub>2</sub> removal prior to freezing. When using oxygen scavengers, major factors affecting isothermal O<sub>2</sub> absorption kinetics are the humidity level, the O<sub>2</sub> concentration, and the gas composition inside the package (Polyakov and Miltz, 2010). The amount of iron present is also a factor for the rate of removal. More iron present will result in more oxygen scavenged.

UV test film # 4 demonstrated in the previous test 5 an  $L^*$  value range of slopes that were only negative over time which is an indication of the product not fading. This test used the UV test film #4 as a control and tests the repeatability of the  $L^*$  value results seen in

test 5. Though previous tests 2 and 5 did not support UV film slowing meat discoloration, the prediction of only negative  $L^*$  values over time for UV test film #4, with the change in the packaging method (flat ham package) and cooler set method (focus on lanes nearest the light source), and using it in combination with another hurdle (oxygen scavenger) warranted revisiting.

#### 4.7.2 – Methods and Material Test 7

Three Beverage Air cooler (Model # LV27 c) with fluorescent bulbs were used in this study. For the cooler set, each cooler contained only one of the three test variables with vertical lanes A, B, and C loaded one sandwich deep on the front lip of the shelf (Figure 4.76).

Date	2/6/2013						
<b>Cooler A</b>				3	2	1	
Current Film + Scavenger				6	5	4	
empty				9	8	7	
lane A				12	11	10	
lane B				15	14	13	
lane C							
<b>Cooler B</b>				3	2	1	
UV Test Film only				6	5	4	
				9	8	7	
				12	11	10	
				15	14	13	
<b>Cooler C</b>				3	2	1	
UV Test Film + Scavenger				6	5	4	
				9	8	7	
				12	11	10	
				15	14	13	

**Figure 4.76** Cooler set up configuration for test 7. Five shelves were utilized for a total of 15 sandwiches per cooler. The color coding is by lane. Light blue represents lane A, Grey lane B, and yellow lane C



Sandwiches were evaluated five times throughout a 28 day refrigerated shelf life for oxygen percentage in the package headspace, Ham  $L^*$  and  $a^*$  color analysis (removed from the package) and visual changes documented in Appendix G. A summary of the sample numbers evaluated and corresponding day in shelf life are listed in Table 4.117.

**Table 4.117** Test 7 sample numbers evaluated and corresponding day in shelf life. This represents one cooler. Each cooler was set up identically

refrigerated Shelf life day	Calendar Date	Sandwiches #'s evaluated		
		Lane A	Lane B	Lane C
Produced on	2/6/2013	sandwiches assembled		
Day 0	2/20/2013	sandwiches placed in refrigeration		
Day 1	2/21/2013	1	2	3
Day 6	2/26/2013	4	5	6
Day 14	3/6/2013	7	8	9
Day 21	3/13/2013	10	11	12
Day 28	3/20/2013	13	14	15

All sandwiches were assembled and placed in MAP (Modified Atmosphere Packaging) with an 80% N<sub>2</sub> / 20% CO<sub>2</sub> blend (Materials and Methods section 3.15) at E.A. Sween Company using a Multivac R530 (Materials and Methods section 3.19). The format of the packaging is the flat ham configuration with ham on top and a package ratio of 1 to 1.8.

The ham, cheese and bread utilized were the same formulations used in test 1-6 (Materials and Methods section 3.1). The ham and cheese was stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly. The bread was stored at room temperature (approximately 21° C) prior to assembly. The length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The

sandwiches spent 14 days in dark frozen storage before evaluation in refrigerated shelf life.

The bottom film used in all three treatments was clear CURLON<sup>®</sup> (Grade 9581-AA) manufactured by Curwood<sup>®</sup> (Osh Kosh, WI). This film is a flexible, formable web for protective packaging of products which are suitable for vacuum and gas applications where low O<sub>2</sub> levels are required. It is recommended for high speed packaging applications where package clarity, outside package C.O.F. (Coefficient Of Friction), uniform formed distribution, and package tightness (i.e. adherence to the product, reducing wrinkles in package) all of which are critical package criteria (Curwood<sup>®</sup>). The oxygen transmission rate (OTR) is O<sub>2</sub> < 0.30 CC per 100 in<sup>2</sup> per 24 hours at 73°F & 0% RH (Relative Humidity). The Moisture Vapor Transmission Rate (MVTR) is MVTR < 0.5 gm H<sub>2</sub>O per 100 in<sup>2</sup> per 24 Hours at 100°F & 90% RH. The starting thickness of the bottom forming film is 8 millimeter. After forming, the minimum pouch thickness is 1 millimeter.

Two top non-forming films were used in this study. The UV film utilized is Belmark UV test film 4 which is 2.96 millimeter thick (48ga UV PET/adhesive/48ga UV PET/adhesive /2.0 mil UV sealant 2) with an OTR of 1 cc/100in<sup>2</sup>/24 hours 73°F/0% RH. In the scavenger only application, the clear top non-forming film used is a lamination of a 50 gauge polyethylene terephthalate (PET) / 200 gauge peelable linear low density polyethylene (LLDPE) co-extrusion (two ply lamination) that is 2.6 millimeter thick, with a OTR rate of <0.5 cc/100 in<sup>2</sup>/24 hour 73°F/0% RH produced by Belmark in De Pere, WI (section 3.3 Methods and Materials). Sandwiches were not labeled to maximize light exposure.

#### **4.7.3 – Oxygen percentages per package over time Test 7**

0% oxygen was achieved in most packages with the exception of two leakers (package with debris in the seal) in week two for the UV only sample (sandwich #4 in red Table 4.118), and the scavenger sachet sample in week one (sandwich #4 in red Table 4.118). The O<sub>2</sub>%, *L*\* and *a*\* results for the leakers are reported but not included in the statistical analysis.

**Table 4.118** Oxygen percentages over time for all treatments - Test 7

	Cooler A - scavenger only			Cooler B - UV test film only			Cooler C - combined		
	lane A	lane B	lane C	lane A	lane B	lane C	lane A	lane B	lane C
Day	Sample S oxygen	Sample S oxygen	Sample S oxygen	Sample UV oxygen	Sample UV oxygen	Sample UV oxygen	Sample SUV oxygen	Sample SUV oxygen	Sample SUV oxygen
1	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000
6	1.52	0.000	0.000	0.000	8.05	0.000	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
max	1.52	0.00	0.00	0.00	8.05	0.00	0.00	0.00	0.00
range	1.52	0.00	0.00	0.00	8.05	0.00	0.00	0.00	0.00

While this demonstrates that the increased capacity scavenger resulted in lower O<sub>2</sub> levels compared to the D-30 cc scavenger from test 5 (O<sub>2</sub>% in test 5 with D-30 cc was 0.20 to 0.41), it is not conclusive as the control (UV film only) also achieved 0.0% oxygen (Table 4.118). This suggests the initial MAP process may have been more efficient for all treatments. The sample with 1.52% O<sub>2</sub> in the scavenger only application was identified as a slow leaker package upon submerging into water. In the case of a leaker package with a scavenger, it is possible that lower O<sub>2</sub> levels may be observed if the leak is slow over time and the scavenger isn't saturated.

#### 4.7.4 –*a*\* scores Test 7

The range of actual *a*\* values over time in this test were 18.05 to 20.95 for the ferrous based scavenger only treatment ( $\Delta a^* = 2.9$ ), 13.89 to 20.57 for the UV film only ( $\Delta a^* = 6.7$ ), and 14.86 to 20.79 for the combined package ( $\Delta a^* = 5.9$ ) (Table 4.119). The variability of actual *a*\* values within all treatments and across all lanes (A, B, and C) over time was not as large as the range of scores demonstrated in Test 6. In Test 6, the reported range for the control package was 3.91 to 19.35 (Table 4.104 in previous section). The leaker package *a*\* values are reported below (Table 4.119 in red), but not included in the statistical slope analysis or in the min / max / range values in Table 4.119). Raw data for *L*\**a*\**b*\* scores is in Appendix G.6.

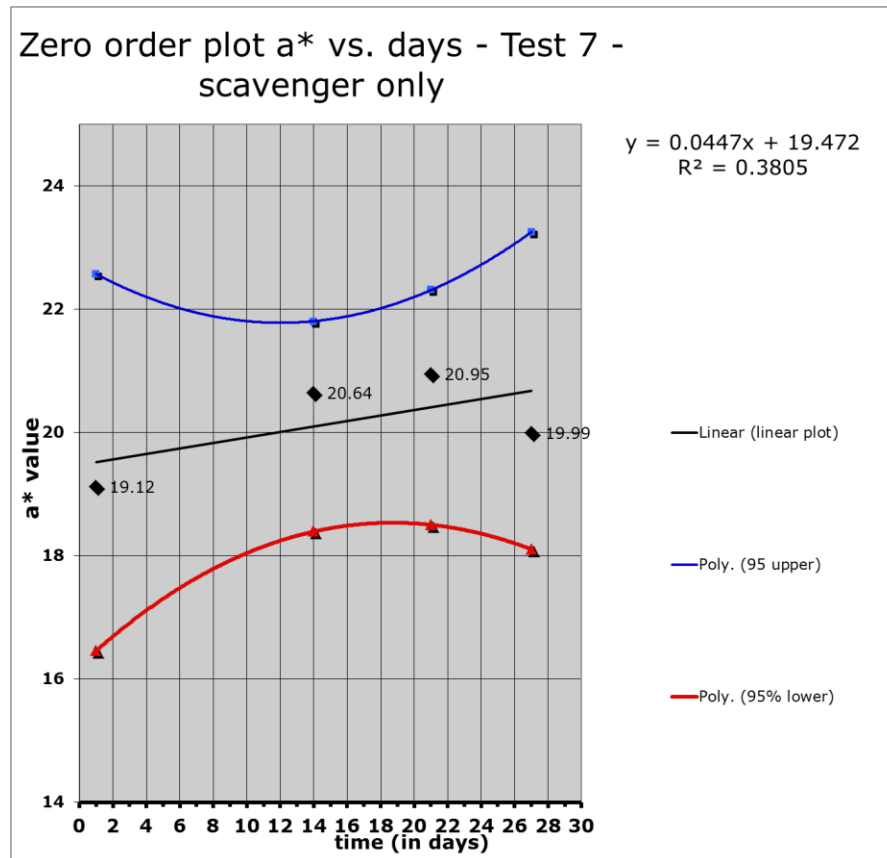
**Table 4.119**  $a^*$  color scores both test and control film test 7. Values in red indicate packages that were identified as leakers (improper seal) with high oxygen in the headspace

	Cooler A - scavenger only			Cooler B - UV test film only			Cooler C - combined		
	lane A	lane B	lane C	lane A	lane B	lane C	lane A	lane B	lane C
Day	Sample S $a^*$	Sample S $a^*$	Sample S $a^*$	Sample UV $a^*$	Sample UV $a^*$	Sample UV $a^*$	Sample SUV $a^*$	Sample SUV $a^*$	Sample SUV $a^*$
1	19.12	19.45	20.15	18.71	17.73	20.57	19.45	20.61	17.56
6	7.10	18.05	19.17	13.89	6.50	20.10	17.60	19.61	17.99
14	20.64	20.63	20.70	18.71	18.04	18.41	18.36	17.05	19.58
21	20.95	18.22	20.16	15.56	18.70	17.36	18.86	14.86	17.93
28	19.99	19.73	19.20	15.82	18.72	19.20	19.26	20.79	20.12
min	19.12	18.05	19.17	13.89	17.73	17.36	17.60	14.86	17.56
max	20.95	20.63	20.70	18.71	18.72	20.57	19.45	20.79	20.12
range	1.83	2.57	1.53	4.82	0.99	3.20	1.85	5.93	2.56

Entering the  $a^*$  values from Table 4.119 above into the kinetics data input sheet (Tables 4.120 – 4.122) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

**Table 4.120** Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the scavenger only sandwiches in Lane A

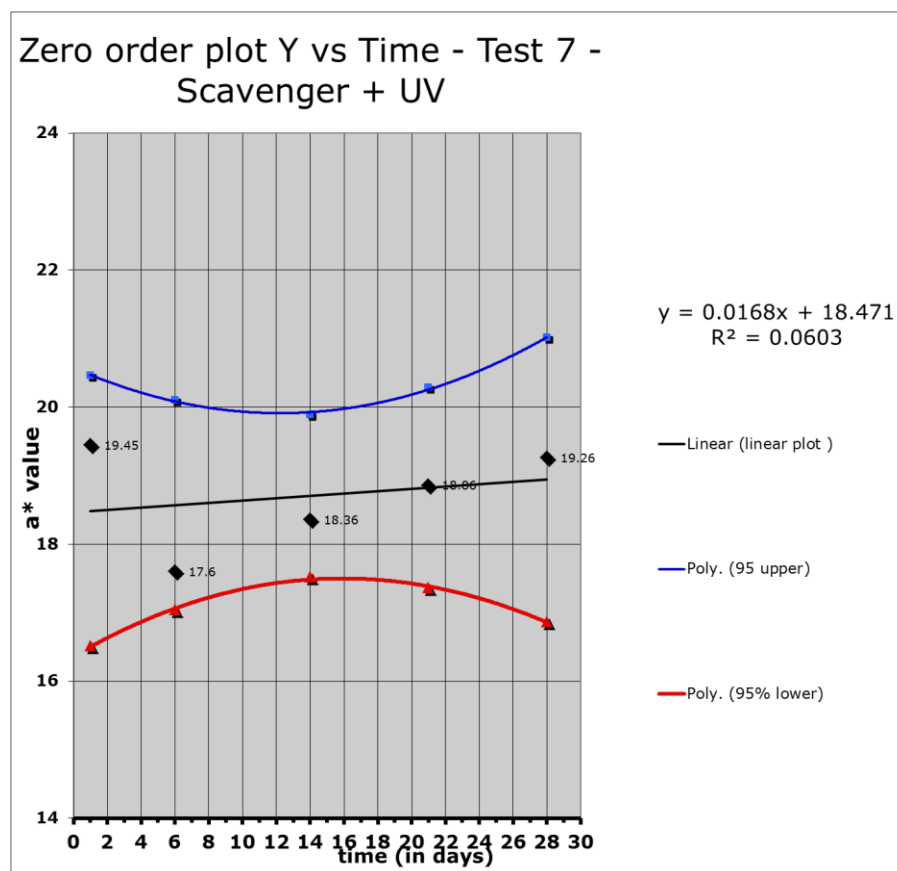
1. Raw Data:															
# data pairs		Total=	4 This is automatically counted												
Y units		a'	Lane A scavenger only												
X units		days													
2. Calculations															
Note after entering Y and X you need to pull down formulas in each column from top to last entry															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
19.12	1.0	365.57	19.12	19.55	1.00	19.12	19.55	0.19	225.00	19.12	1.00	22.65	16.45	6.20	19.55
20.64	14.0	426.01	20.64	20.09	196.00	20.64	20.09	0.30	4.00	288.96	196.00	21.84	18.34	3.50	20.09
20.95	21.0	438.90	20.95	20.38	441.00	20.95	20.38	0.32	25.00	439.95	441.00	22.30	18.46	3.84	20.38
19.99	28.0	399.60	19.99	20.67	784.00	19.99	20.67	0.47	144.00	559.72	784.00	23.36	17.99	5.37	20.67
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
STATISTICS															
slope= 0.0416															
intercept= 19.5097															
rsq= 0.3504															
± 95% slope 0.1722															
k upper 0.2137															
k lower -0.1306															
Standard Error 0.80															
Sum (yi-yes) 2665.67															
n 4.00															
t 95%, 2, n-2 4.30															
x average = 16.00															
Sum (xi-xav) 2190.00															
(Sum x)^2 4096.00															
Sum (y^2) 1630.09															
sum y 80.70															
Sum (xi*yi) 1307.75															
sum x 64.00															
sum (X^2) 1422.00															
Equations															
Y = 19.5097 + 0.0416 * time															



**Figure 4.77** Test 7: Scavenger only Ham Zero order plot of  $a^*$  vs. time in lane A (28 days) with 95 % confidence limits calculation

**Table 4.121** Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for Scavenger + UV packaging sandwiches in Lane A

1. Raw Data:															
# data pairs Total=		5 This is automatically counted													
Y units		a" Lane A - Scavenger + UV													
X units		days													
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry $(y_i - y_{est})^2$															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	$(y_i - y_{est})^2$	$(x_i - x_{ave})^2$	$x_i * y_i$	X^2	y 95%UL	y 95%LL	Delta	predicted average
19.45	1.0	378.30	19.45	18.49	1.00	19.45	18.49	0.93	169.00	19.45	1.00	20.46	16.51	3.95	18.49
17.6	6.0	309.76	17.60	18.57	36.00	17.60	18.57	0.94	64.00	105.60	36.00	20.11	17.04	3.07	18.57
18.36	14.0	337.09	18.36	18.71	196.00	18.36	18.71	0.12	0.00	257.04	196.00	19.89	17.52	2.38	18.71
18.86	21.0	355.70	18.86	18.82	441.00	18.86	18.82	0.00	49.00	396.06	441.00	20.28	17.36	2.92	18.82
19.26	28.0	370.95	19.26	18.94	784.00	19.26	18.94	0.10	196.00	539.28	784.00	21.02	16.87	4.15	18.94
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	$(y_i - y_{est})^2$	$(x_i - x_{ave})^2$	$X_i * Y_i$	X^2	y 95%UL	y 95%LL	Delta	predicted average
slope=				0.0168								Standard Error		0.84	
intercept=				18.4714								Sum (yi-yes)		2049.25	
rsq=				0.0603								n		5.00	
± 95% slope				0.1215								t 95%,2,n-2=		3.18	
k upper				0.1382								x average =		14.00	
k lower				-0.1047											
Equations												Sum (xi-xav)		1654.00	
Y = 18.4714 + 0.0168 * time												(Sum x)^2		4900.00	
												Sum(y^2)		1751.80	
												sum y		93.53	
												Sum (xi*yi)		1317.43	
												sum x		70.00	
												sum (X^2)		1458.00	

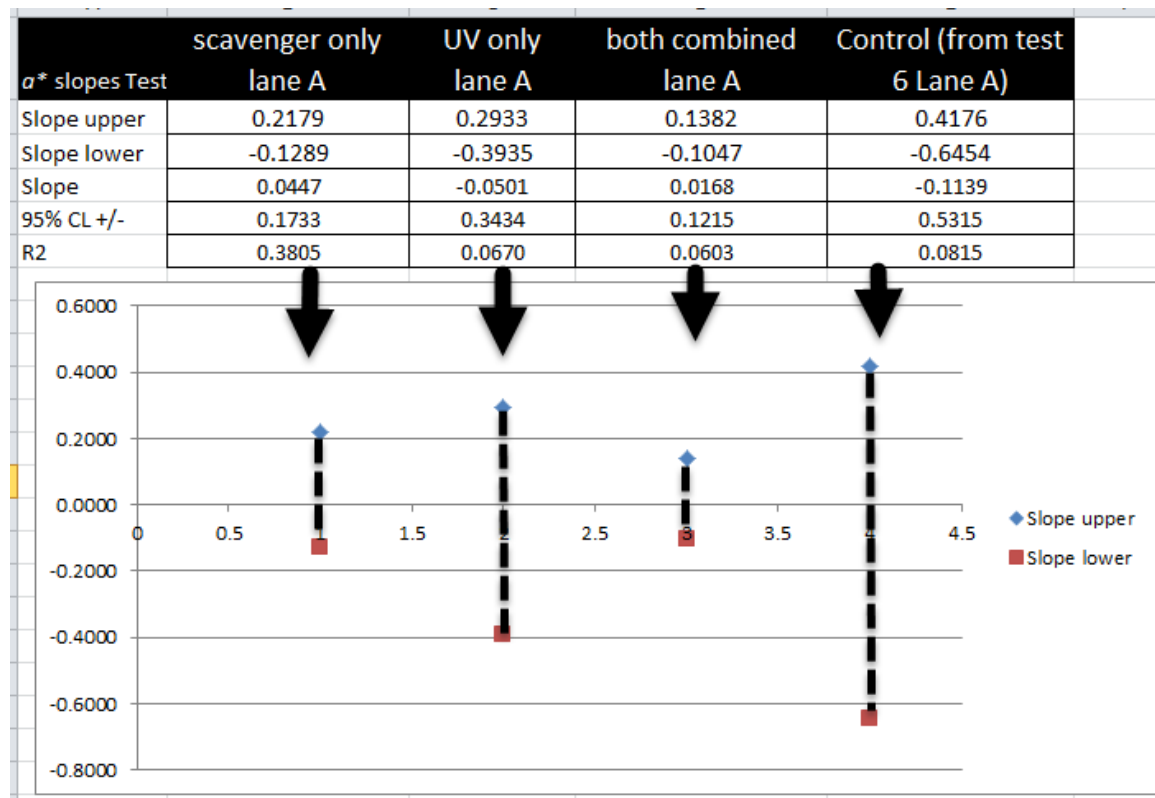


**Figure 4.78** Test 7: Scavenger + UV packaging Ham Zero order plot of  $a^*$  vs. time in lane A (28 days) with 95 % confidence limits calculation



Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments is provided in Table 4.123. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 4.123**  $a^*$  rate constant ( $k$ ) upper and lower for all applications in Test 7 lane A as established by Labuza's Reaction kinetics shelf life model. The control sample results from Test 6 in lane A is included for reference



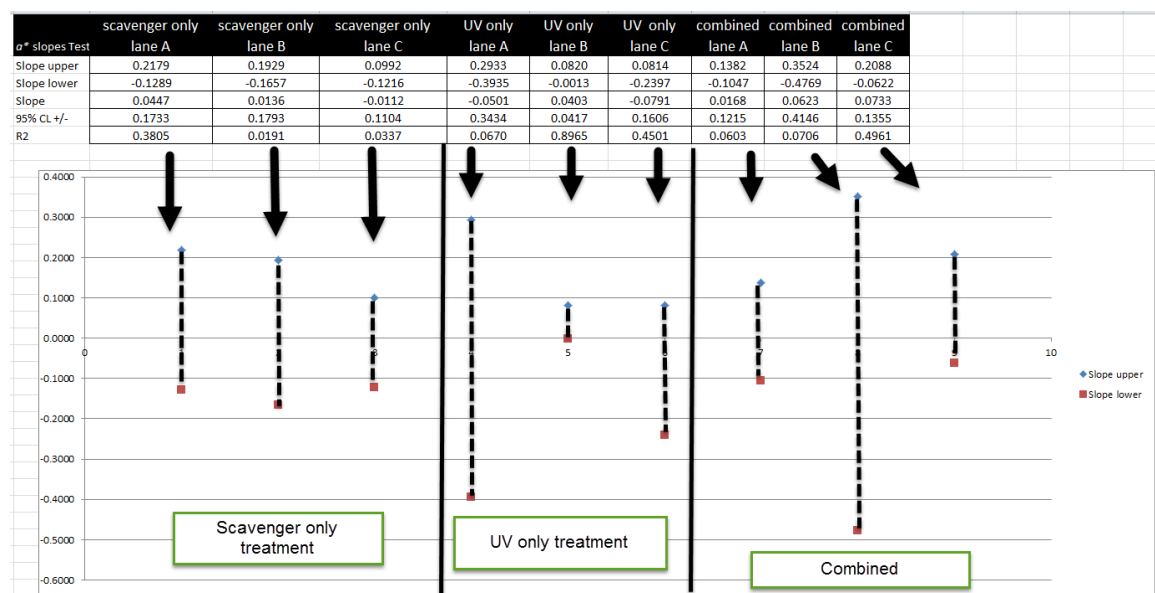
While not statistically different, the similarity in outcomes for predicted slopes for the scavenger only and scavenger plus UV film suggest that the scavenger is a the common key component creating less variability in the range of  $a^*$  values over time, with a greater likelihood of positive slopes (increasing redness) than the UV only application. The UV



only treatment has a broader range (less predictable) of outcomes, with a greater chance of negative slope over time. All treatments show a smaller range of potential outcomes when compared to the control package in lane A from test 6, but are not statistically different from each other (Table 4.123). The fit of data (as measured by  $R^2$ ) was again low for all treatments due to the limited number of samples and high variability in  $a^*$  color score results over time which is attributed to both the starting ham color variation (due to the formulation) and changes due to photo-oxidation during shelf life. The trend line for each treatment shows an overall positive slope for both treatments with the  $O_2$  scavenger, and a negative slope for the UV only film (Figures 4.77-4.79)

When comparing kinetic reaction slope outcomes in lanes B and C across all treatments, there also was no statistical difference between lanes A-C (Table 4.124), which supports the findings from Test 6.

**Table 4.124**  $a^*$  Rate constant (k) upper and lower for all treatments and all lanes in Test 7 as established by Labuza's Reaction kinetics shelf life model



In this test, the UV only treatment showed greater variability in lane A compared to lanes B and C, where the combined UV film and  $O_2$  scavenger treatment had greater variability in lane B compared to lanes A and C. The scavenger only treatment showed the greatest consistency of outcomes across all lanes (Table 4.124). The kinetics data input sheet for all treatments in lanes B and C can be found in the Appendix (G.7 – G.12).

#### 4.6.5 – $L^*$ scores Test 7

The variability of  $L^*$  values across all treatments over time was also substantial with a range of scores for the scavenger only from 56.58 to 61.44, UV film only from 55.93 to 62.82, and the combined package at 57.22 to 65.24 (Table 4.125). This is also similar to the range found in the control packages in Test 6 (57.36 to 65.47 see Table 4.110).

**Table 4.125**  $L^*$  values for lanes A, B, and C for all treatments in Test 7. Red highlight indicates leaker values

	Cooler A - scavenger only			Cooler B - UV test film only			Cooler C - combined		
	lane A	lane B	lane C	lane A	lane B	lane C	lane A	lane B	lane C
Day	Sample S $L^*$	Sample S $L^*$	Sample S $L^*$	Sample UV $L^*$	Sample UV $L^*$	Sample UV $L^*$	Sample SUV $L^*$	Sample SUV $L^*$	Sample SUV $L^*$
1	61.03	60.20	58.85	61.97	61.87	59.04	60.20	57.34	63.24
6	59.82	61.44	60.68	59.64	63.10	58.13	61.25	59.71	65.24
14	57.95	58.88	58.70	57.68	59.81	60.10	62.30	63.51	58.42
21	56.58	59.68	59.40	58.32	61.30	62.82	57.22	62.95	59.92
28	58.50	59.67	59.24	59.48	58.06	55.93	60.97	57.62	59.38
min	56.58	58.88	58.70	57.68	58.06	55.93	57.22	57.34	58.42
max	61.03	61.44	60.68	61.97	61.87	62.82	62.30	63.51	65.24
range	4.44	2.55	1.98	4.30	3.81	6.88	5.08	6.17	6.82

The range of actual  $L^*$  slope values for the scavenger treatment is greatest in lane A (4.44), with narrower ranges for lanes B (2.55) and C (1.98). This demonstrates greater variability again for lane A; similar to what was observed with the control package in the previous test 6. However, the other two treatments in this test show greater  $L^*$  ranges in lane C (6.88; 6.82), followed by B and A (Table 4.125 above).

Entering the  $L^*$  values from Table 4.125 above into the kinetics data input sheet (Tables 4.126 – 4.128) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey pigment or concentration of pigments (which is also a result of moisture loss).

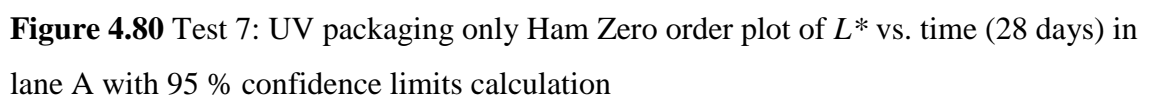
1. Raw Data:		4 This is automatically counted	
# data pairs Total=	L *	Lane A - UV film only	
Y units	days		
X units			

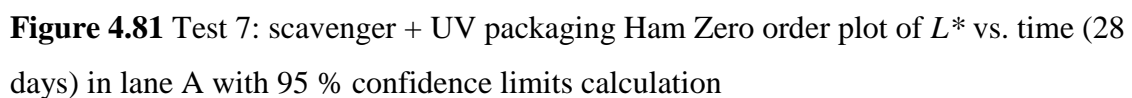
  

STATISTICS															
2. Calculati Note after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yes)*^2															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average	
61.97	1.0	3840.69	61.97	60.86	1.00	61.97	60.86	1.25	225.00	61.97	1.00	68.02	53.69	14.33	60.86
57.68	14.0	3326.60	57.68	59.56	196.00	57.68	59.56	3.55	4.00	807.47	196.00	63.61	55.51	8.10	59.56
58.32	21.0	3400.83	58.32	58.86	441.00	58.32	58.86	0.30	25.00	1224.65	441.00	63.30	54.42	8.88	58.86
59.48	28.0	3537.47	59.48	58.17	784.00	59.48	58.17	1.72	144.00	1665.35	784.00	64.37	51.96	12.42	58.17
		0.00	0.00	60.95	0.00	0.00	60.95	3715.49	256.00	0.00	0.00	68.46	53.45	15.00	60.95
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
slope=				-0.0996				Standard Er				1.85			
intercept=				60.9548				Sum (yi-yes)				26015.23			
rsq=				0.3669				n				4.00			
± 95% slope				0.3979				t 95%,2,n-2				4.30			
k upper				0.2983				x average =				16.00			
k lower				-0.4975											
								Sum (xi-xav				2190.00			
								(Sum x)^2				4096.00			
								Sum(y^2)				14105.60			
								sum y				237.44			
								Sum (xi*yi)				3759.44			
								sum x				64.00			
								sum (X^2)				1422.00			

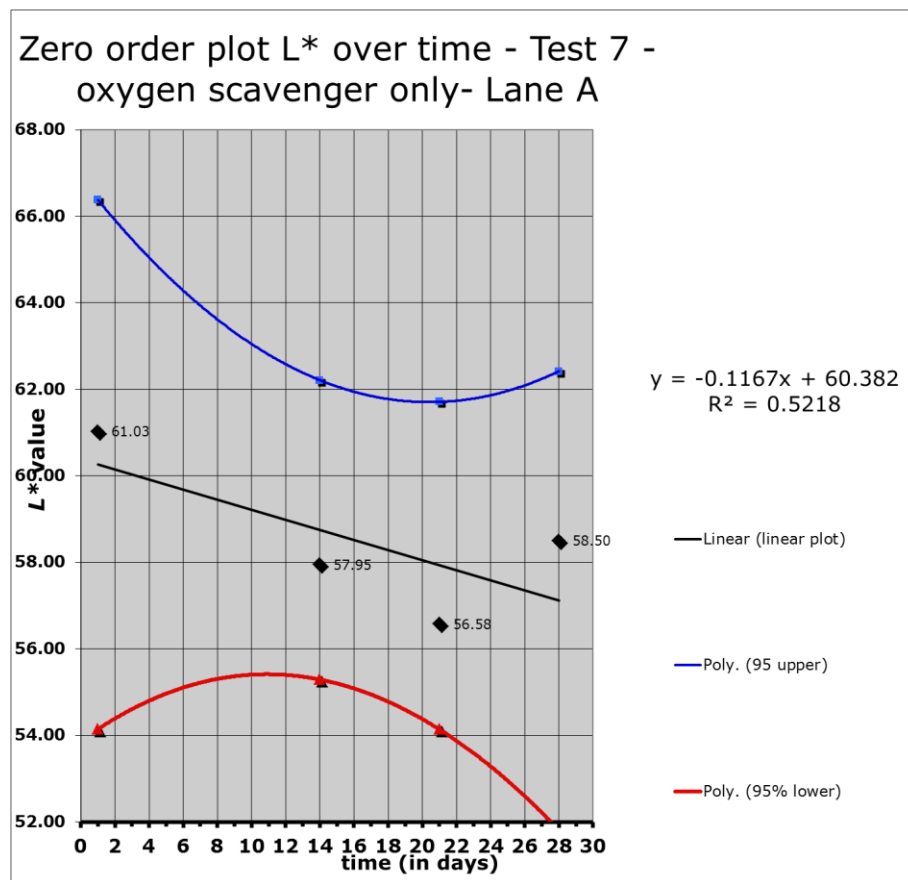
Equations		
Y =	60.9548	-0.0996 * time



[illegible]

**Table 4.128** Test 7  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for scavenger only packaging sandwiches in Lane A

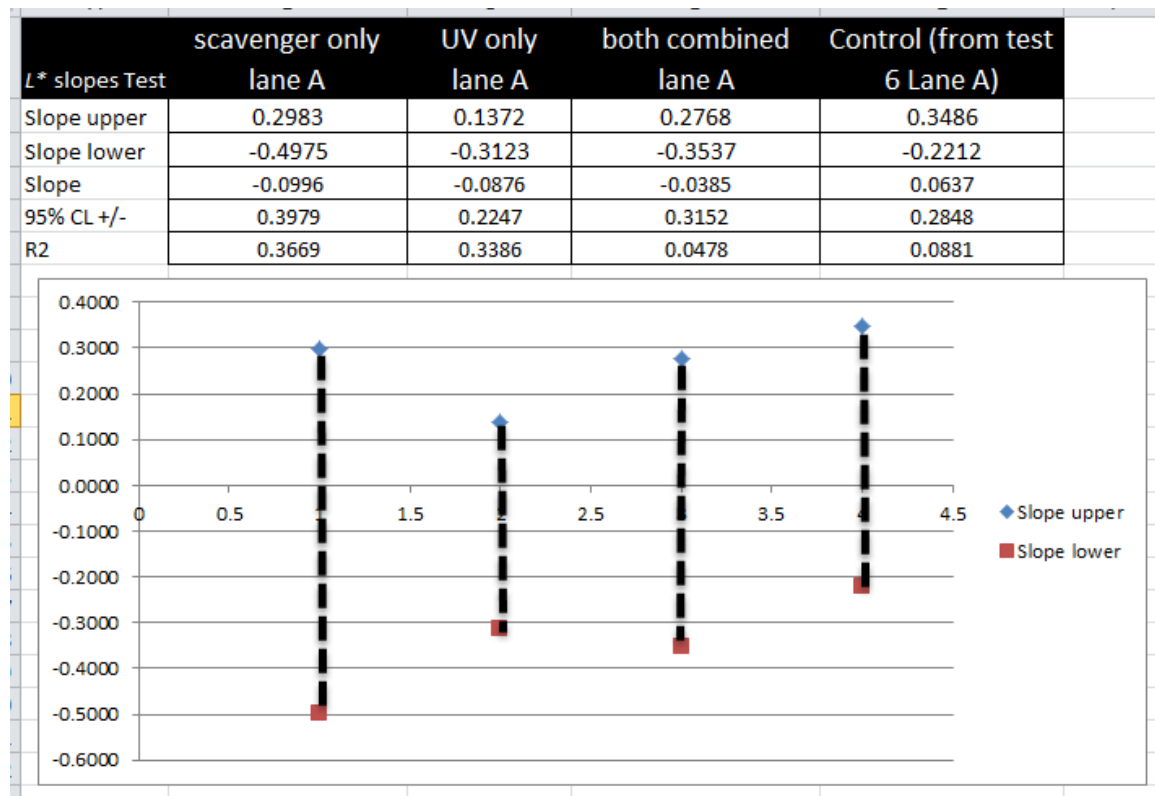
1. Raw Data:															
# data pairs Total=		4 This is automatically counted													
Y units		L* Lane A - UV oxygen scavenger sachet only													
X units		days													
STATISTICS															
2. Calculati Note after entering Y and X you need to pull down formulas in each column from top to last entry															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	X^2	y 95%UL	y 95%LL	Delta	predicted average		
61.03	1.0	3724.66	61.03	60.27	1.00	61.03	60.27	0.58	225.00	61.03	1.00	66.38	54.15	12.24	60.27
57.95	14.0	3358.20	57.95	58.75	196.00	57.95	58.75	0.64	4.00	811.30	196.00	62.20	55.29	6.91	58.75
56.58	21.0	3201.30	56.58	57.93	441.00	56.58	57.93	1.83	25.00	1188.18	441.00	61.72	54.14	7.58	57.93
58.50	28.0	3422.25	58.50	57.11	784.00	58.50	57.11	1.92	144.00	1638.00	784.00	62.42	51.81	10.60	57.11
		0.00	0.00	60.38	0.00	0.00	60.38	3646.03	256.00	0.00	0.00	66.79	53.98	12.81	60.38
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
slope=		-0.1167													
intercept=		60.3823													
rsq=		0.5218													
± 95% slope		0.3397													
k upper		0.2230													
k lower		-0.4564													
Equations															
Y = 60.3823 - 0.1167 * time															
Standard Error 1.58															
Sum (yi-yes) 25527.15															
n 4.00															
t 95%,2,n-2= 4.30															
x average = 16.00															
Sum (xi-xav) 2190.00															
(Sum x)^2 4096.00															
Sum(y^2) 13706.41															
sum y 234.06															
Sum (xi*yi) 3698.51															
sum x 64.00															
sum (X^2) 1422.00															



**Figure 4.82** Test 7: scavenger only Ham Zero order plot of  $L^*$  vs. time (28 days) in lane A with 95 % confidence limits calculation

Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges (+k for lightening over the shelf life, - k for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.129.

**Table 4.129**  $L^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in test 7 as established by Labuza' Reaction kinetics shelf life model



The scavenger only treatment demonstrates the greatest potential for fade over time in this study (positive slope), followed by the scavenger with UV film treatment. The UV only treatment demonstrated the least potential for fade over time. However, there is overlap of potential outcomes for all treatments, which makes the performance for each not statistically different. Compared to the control package from Test 6 (Table 4.129

above), all three treatments performed similarly and are not statistically different. While in test 5 the UV test film #4 demonstrated only negative slopes over time, this test found the potential for positive slopes. Because the results were not repeated, the conclusion is that none of the treatments create an advantage for the light or dark perception of the ham.

#### **4.7.6 – Visual appearance of the ham Test 7**

With the exception of the leaker package (1.52%), all O<sub>2</sub> scavenger only samples were visually pink compared to the UV only film package which showed signs of significant discoloration over time in lanes A and B. (Appendix G.1-G.5) The scavenger with UV film also turned grey at day six of the refrigerated shelf life despite having 0% oxygen (Appendix G.2 – Lane B). This leads to the speculation that the package potentially had residual oxygen present at the start of refrigeration that was consumed in the formation of metmyoglobin by day 6.

#### **4.7.7 – Cooler temperatures Test 7**

The coolers utilized were the same coolers used in Tests 2-4. The settings were not adjusted. Temperature was not tracked in this study. In Tests 2-4, the coolers established a consistent average temperature between 0.0 – 1.0 C°.

#### **4.7.8 – Conclusions Test 7**

The scavenger treatments did result in maintaining 0% oxygen during the refrigerated shelf life in all packages and visually did not demonstrate discoloration in the scavenger only treatment. While this test demonstrated that the increased capacity of the D-50 cc scavenger was more effective in removing oxygen compared to D-30 cc (test 5 where use of D-30 cc resulted in no packages achieving 0% O<sub>2</sub>). The UV only samples in this test also achieved 0% suggesting this may be the outcome of equipment variability (i.e. the MAP equipment for this batch of products was more effective in removing oxygen). This outcome for D-50 cc was also similar to test 4 where use of the O<sub>2</sub> scavenger D-30 cc

resulted in only 2 out of 20 packages with residual oxygen at the time of evaluation. For these reasons, a repeated test of D-50 cc is needed to verify consistency.

Of concern in this test was development of visual discoloration in the combined option (O<sub>2</sub> scavenger + UV film) at day 6 despite having 0% O<sub>2</sub>. This suggests that the package may not have achieved 0% at the start of refrigerated shelf life or the combination of the UV protecting film with the scavenger created a countering affect.

While both the scavenger sachet applications (scavenger only and UV film + scavenger) demonstrated less volatility for  $a^*$  outcomes over time compared to UV film only, the kinetics reaction tool found the  $a^*$  value predicted outcomes for all treatments to not be statistically different from each other.

The UV film did not repeat the  $L^*$  value results achieved in test 5, and verified that UV film alone or in combination with another hurdle is not creating a benefit.

The success of others with using oxygen scavengers to protect cured meat color continues to make this a strong area of interest. Hormel in the late 1980's observed similar issues as they transitioned pepperoni from vacuum pack to Modified Atmosphere Packaging (MAP). Because of MAP equipment limitations and entrained oxygen in the pepperoni (oxygen incorporated into the meat from the blending process and not completely removed in the vacuum mixer), pepperoni was discolored because of residual oxygen still in the package and light exposure. Addition of an oxygen scavenger absorbed head space oxygen and prevented pepperoni discoloration (Miller, Hormel<sup>®</sup>). Key differences in the success of O<sub>2</sub> scavengers with pepperoni include storage temperatures (ranging from refrigerated to room temperature), and a different dynamic for air flow in the package (as the pepperoni slices are more free flowing).

Anderson and Rasmussen also found that for refrigerated sliced ham, visual differences in discoloration were observed in scavenger packages as compared to MAP only (as judged by a 5 member sensory panel), and with  $\Delta a^*$  differences of as much as 4 points observed throughout the shelf life (Measured with a chromameter and using the Hunter lab Lab scale) (Anderson and Rasmussen, 1992). Limbo et al. experienced a similar outcome with fresh beef steaks packaged with a scavenger. Without a scavenger, the fresh beef steaks had irreversible discoloration within 7 days. With the scavenger, the product maintained a red appearance (Limbo et al., 2013).



## **4.8 - Test 8 Non-ferrous based oxygen scavenging film**

### **4.8.1 – Test 8 overview**

The purpose of this test was to determine if a non-ferrous based oxygen scavenging film (Cryovac<sup>®</sup> OS 2030 film) is effective in slowing meat discoloration over time.

The benefits, if effective, are to eliminate the need for a foreign object in the package (oxygen scavenger sachet) and not requiring adjustments to metal detection settings as is needed with a ferrous based scavenger pack. Metal detection happens post sealing of the package and outside of a Ready To Eat (RTE) area. This is a common design for most manufacturing facilities, and the disadvantages are line shut down to investigate cause, and any false positives from metal detection become difficult to rework after a product is outside of the ready to eat space.

The non-ferrous scavenging film is a multilayer coextruded film that incorporates both oxygen barrier and oxygen scavenging in the middle layers. (Cryovac<sup>®</sup>) Based on a system of proprietary technologies, this polymer based method reduces oxygen levels in MAP applications during refrigerated storage. The mechanism of scavenging is accomplished when ethylene methyl acrylate cyclohexene methanol is exposed to UV light. (See Figure 3.6 in Methods and materials) The ring is able to oxidize with sufficient UV energy with the presence of a catalyst (cobalt). Optimal performing temperature range is 3.3 – 21°C. (Cryovac<sup>®</sup>) The film is produced by Cryovac<sup>®</sup> in Duncan, SC.

### **4.8.2 – Methods and Materials Test 8**

Two Beverage Air cooler (Model # LV27 c) with fluorescent lighting (Buyers Choice cool white 32 watt fluorescent bulb Methods and Materials 3.18) were used in this study (the coolers designated “A” and “C” from previous studies). For the cooler set, each cooler contained only one test variable, with vertical lanes A, B, and C loaded one sandwich deep on the front lip (Figure 4.83).

Meat Discoloration Study 8				
Cooler A		3	2	1
Control Current Film		6	5	4
		9	8	7
		12	11	10
		15	14	13
		18	17	16
Cooler C		3	2	1
Cryovac Test Film		6	5	4
		9	8	7
		12	11	10
		15	14	13
		18	17	16

**Figure 4.83** Cooler set up configuration for test 8. Six shelves were utilized for a total of 18 sandwiches per cooler. The color coding is by lane. Light blue represents lane A, light green for lane B, and yellow lane C

Six sandwiches (3 per treatment – lanes A, B, and C) were removed on each designated day and evaluated six times throughout a 30 day refrigerated shelf life for oxygen percentage in the package headspace, Ham  $L^*$  and  $a^*$  color analysis (removed from the package) and visual evaluation (photographs documented in Appendix H.1-H.6). A summary of the sample numbers evaluated and corresponding day in shelf life are listed in Table 4.130.

**Table 4.130** Test 8 sample numbers evaluated and corresponding day in shelf life

Date	Shelf life Day	Control samples, number evaluated			Non-ferrous based samples, number evaluated		
		Lane A	Lane B	Lane C	Lane A	Lane B	Lane C
12/18/2013	sandwiches assembled, packaged and frozen						
1/6/2014	refrigerated shelf life begins						
1/7/2014	1	1	2	3	1	2	3
1/8/2014	2	4	5	6	4	5	6
1/9/2014	3	7	8	9	7	8	9
1/10/2014	4	10	11	12	10	11	12
1/13/2014	7	13	14	15	13	14	15
2/5/2014	30	16	17	18	16	17	18

The ham, cheese, and bread utilized were consistent with Tests 1-7 and are described in methods and materials section 3.1. Each sandwich component used was from the same production lot to minimize batch to batch variability. The ham and cheese were stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly. The bread was stored at room temperature (approximately 21° C) prior to assembly. The length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The age of the ham at the time of packaging was 26 days old. The shelf life of the ham in the log form and casing is 120 days from the day of manufacture. In practice the quality standard are that the ham can be no older than 60 days at the time of sandwich assembly. This practice is followed to allow for adequate remaining shelf life on the ham at the time of freezing and before display at the store.

The control packaging used is as described in section 3.3 (Belmark clear non-forming film) and 3.4 (Curwood clear forming film). For the test packaging, Cryovac<sup>®</sup> top (non-forming) film is used in conjuncture with a Cryovac<sup>®</sup> forming film. The bottom forming film contains an oxygen barrier layer with oxygen scavenging polymer blended into the middle layer barrier resin for an additional level of oxygen ingress protection (Methods and Materials section 3.7). The Cryovac<sup>®</sup> bottom forming film does not scavenge headspace oxygen and does not require UV activation because it is extruded in the active form. The oxygen Permeability of the bottom forming film is < 1.0 cc STP/(24 hrs., m<sup>2</sup> atm)@ 73°F., 0% RH (Appendix H.9). The Cryovac top non-forming film is based on a system of proprietary technologies, this polymer based method reduces oxygen levels in MAP applications. Scavenging begins when a patented UV light triggering unit, installed on the packaging line, activates the film. The scavenging polymer is incorporated into the package and is invisible to the consumer. The Passive Oxygen Permeability of the top non-form film is 2 cc STP/(24 hrs., m<sup>2</sup> atm)@ 73°F., 0% RH (Appendix H.8).

All sandwiches were assembled and placed in MAP (Modified Atmosphere Packaging) with an 80% N<sub>2</sub> / 20% CO<sub>2</sub> blend (Materials and Methods section 3.15) at E.A. Sween Company using a Multivac R530 (Materials and Methods section 3.19). The format of the packaging for both treatments is the flat ham configuration with ham on top and a

package ratio of 1 to 1.8. The packaged sandwiches were placed in dark frozen storage for 18 days before the start of the refrigerated shelf life.

#### 4.8.3 – Oxygen (O<sub>2</sub>) percentages per package Test 8

The maximum and range (maximum minus minimum value) of O<sub>2</sub> percentages achieved in both the control and test film are very similar (Table 4.131)

**Table 4.131** Oxygen percentages in the headspace for control and test film over time (all lanes) Test 8. Red indicates packages identified as leakers.

day	Control lane A	Control lane B	Control lane C	Oxygen scavenging film lane A	Oxygen scavenging film lane B	Oxygen scavenging film lane C
	O <sub>2</sub> %	O <sub>2</sub> %	O <sub>2</sub> %	O <sub>2</sub> %	O <sub>2</sub> %	O <sub>2</sub> %
1	0.075	0.172	0.241	0.020	0.070	0.090
2	0.091	0.053	0.063	0.051	0.132	0.160
3	0.021	13.500	0.071	0.102	12.100	0.106
4	0.002	0.029	0.268	0.037	0.092	0.089
7	0.000	0.006	0.024	0.000	0.038	19.100
30	0.000	0.000	0.000	0.000	0.000	0.000
min	0.00	0.00	0.00	0.00	0.00	0.00
max	0.09	0.17	0.27	0.10	0.13	0.16
range	0.09	0.17	0.27	0.10	0.13	0.16

Given that the non-ferrous based scavenger did not reach 0% O<sub>2</sub> in the package during the first 4 days, and the similar maximum values and ranges in O<sub>2</sub> values compared to the control, it is reasonable to conclude that the oxygen scavenging film was not effective in reducing O<sub>2</sub> levels. Both the control and non-ferrous packages reached 0% oxygen in lane A at day 7. Given the visual discoloration in both products at day 7(Appendix H.5), the oxygen was likely consumed completely in the photo-oxidation reaction.

#### 4.8.4 – Ham $a^*$ scores Test 8

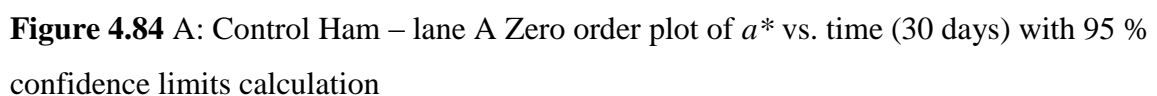
The variability of  $a^*$  values within and across all lanes over time was large for both treatments with a range of scores from 10.69 to 18.20 for the non-ferrous based scavenger and 13.77 to 18.52 for the control package (Table 4.132). The leaker packages  $a^*$  values are reported below (Table 4.132 in red), but are not included in the statistical comparison or in the minimum and maximum values in the table.

**Table 4.132**  $a^*$  color scores both test and control film Test 8. Values in red indicate packages that were identified as leakers (improper seal) resulting in high oxygen in the headspace

day	Control lane A $a^*$	Control lane B $a^*$	Control lane C $a^*$	Oxygen scavenging film lane A $a^*$	Oxygen scavenging film lane B $a^*$	Oxygen scavenging film lane C $a^*$
1	15.82	17.72	17.96	16.30	18.20	18.19
2	14.14	15.41	18.01	14.44	15.66	16.67
3	16.96	7.56	15.73	13.19	16.47	17.57
4	13.77	16.09	14.81	16.93	17.60	17.12
7	13.98	12.60	16.66	15.70	16.80	8.63
30	14.20	16.25	18.52	10.69	14.84	17.33
min	13.77	12.60	14.81	10.69	14.84	16.67
max	16.96	17.72	18.52	16.93	18.20	18.19
range	3.19	5.12	3.71	6.23	3.36	1.52

The largest range of  $a^*$  values over time occurred with the non-ferrous based scavenger (range = 6.23 in lane A), while the control had range = 5.2 in lane B). Compared to the previous test (7), the ferrous based scavenger had its largest  $a^*$  range = 2.57 in lane B, with the UV only treatment  $a^*$  range = 4.82, and the combined UV film + ferrous based scavenger  $a^*$  range = 5.93 (Table 4.119 in previous section). To date, the ferrous based scavenger has produced the smallest range of variability for  $a^*$  color scores. While these ranges in previous tests have been proven not to be statistically different using the reactions kinetics tool, a narrower range of outcomes with a greater chance of  $a^*$  positive slope (increasing redness) over negative slope (decreasing redness) has positive implications.

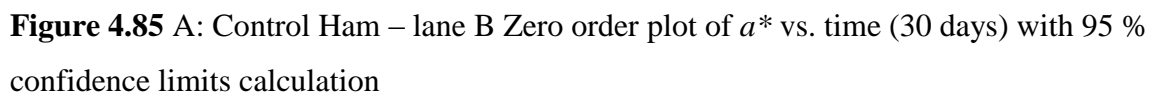
Entering the  $a^*$  values from Table 4.132 above into the kinetics data input sheet (Tables 4.133 – 4.138) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

[illegible]

1. Raw Data:																	
# data pairs	Total=	5		This is automatically counted													
Y units	a*	Lane B															
X units	days																

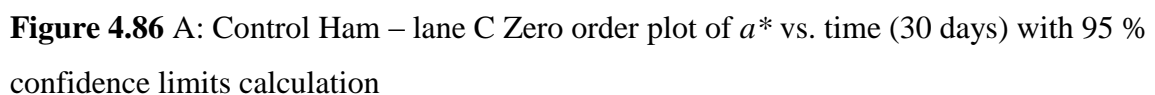
  

STATISTICS																
2. Calculati	Note after entering Y and X you need to pull down formulas in each column from top to last entry	rd(yi-yes)	*2	(xi-xave)*2	xi*yi	X*2	y 95%UL	y 95%LL	Delta	predicte						
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)	*2	(xi-xave)*2	Xi*Yi	X*2	y 95%UL	y 95%LL	Delta	predicte
17.72	1.0	314.12	17.72	15.59	1.00	17.72	15.59	4.53	60.84	17.72	1.00	19.41	11.78	7.63	15.59	
15.41	2.0	237.57	15.41	15.60	4.00	15.41	15.60	0.03	46.24	30.83	4.00	19.25	11.94	7.31	15.60	
16.09	4.0	258.89	16.09	15.60	16.00	16.09	15.60	0.24	23.04	64.36	16.00	18.99	12.22	6.77	15.60	
12.60	7.0	158.76	12.60	15.61	49.00	12.60	15.61	9.06	3.24	88.20	49.00	18.75	12.47	6.27	15.61	
16.25	30.0	264.06	16.25	15.67	900.00	16.25	15.67	0.34	449.44	487.50	900.00	22.49	8.85	13.64	15.67	
		0.00	0.00	15.59	0.00	0.00	15.59	243.12	77.44	0.00	0.00	19.58	11.60	7.98	15.59	
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes)	*2	(xi-xave)*2	Xi*Yi	X*2	y 95%UL	y 95%LL	Delta	predicte
		slope=		0.0026								Standard Er		2.18		
		intercept=		15.5922								Sum (yi-yes)		1472.89		
		rsq=		0.0003								n		5.00		
		± 95% slope		0.2866								t 95%, 2,n-2=		3.18		
		k upper		0.2892								x average =		8.80		
		k lower		-0.2840												
Equations																
		Y = 15.5922		0.0026		* time										



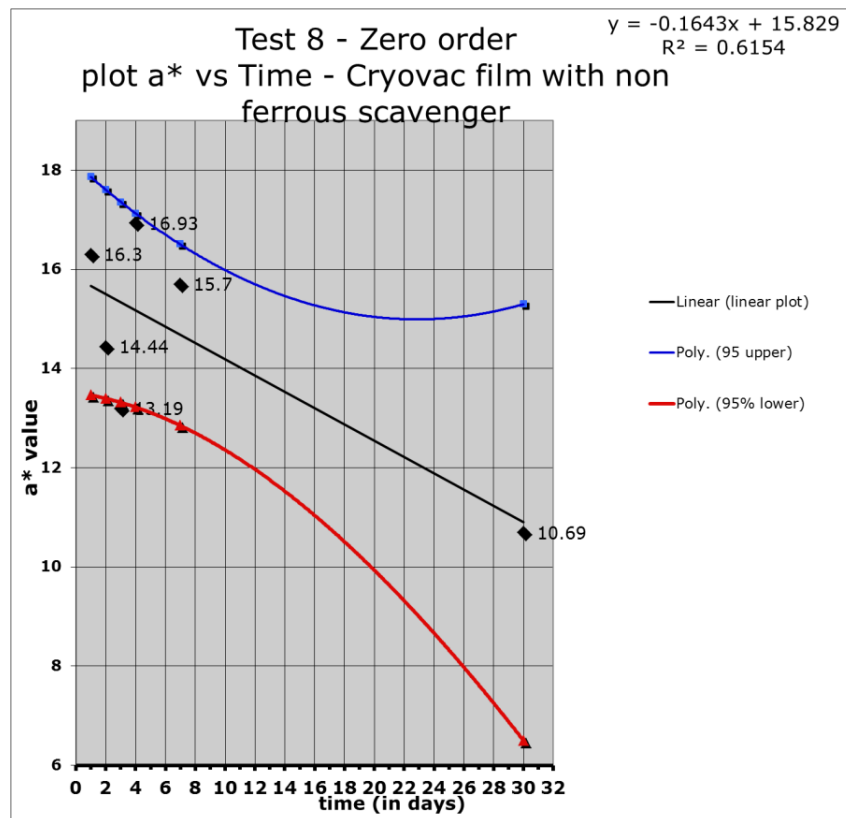


1. Raw Data:																			
# data pairs	Total=	6	This is automatically counted																
Y units	a"		Lane C																
X units	days																		
										STATISTICS									
2. Calculations										Note after entering Y and X you need to pull down formulas in each column from top to last entry row									
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predictive average				
17.96	1.0	322.56	17.96	16.54	1.00	17.96	16.54	2.00	46.69	17.96	1.00	18.56	14.53	4.03	18.54				
18.01	2.0	324.24	18.01	16.60	4.00	18.01	16.60	1.97	34.03	36.01	4.00	18.53	14.68	3.85	16.60				
15.73	3.0	247.43	15.73	16.66	9.00	15.73	16.66	0.87	23.36	47.19	9.00	18.51	14.81	3.70	16.66				
14.81	4.0	219.24	14.81	16.72	16.00	14.81	16.72	3.67	14.69	59.23	16.00	18.50	14.94	3.57	16.72				
16.66	7.0	277.67	16.66	16.90	49.00	16.66	16.90	0.06	0.69	116.64	49.00	18.57	15.23	3.35	16.90				
18.52	30.0	342.87	18.52	18.25	900.00	18.52	18.25	0.07	491.36	555.50	900.00	22.28	14.23	8.05	18.25				
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predictive average				
slope=												0.0590							
intercept=												16.4854							
rsq=												0.1974							
± 95% slope												0.1652							
k upper												0.2242							
k lower												-0.1063							
Equations																			
Y =												16.4854		0.0590		* time			
Standard Error												1.47							
Sum (yi-yes)												1367.47							
n												6.00							
t 95%, 2, n-2=												2.78							
x average =												7.83							
Sum (xi-xav)												917.64							
(Sum x)^2												2209.00							
Sum(y^2)												1734.01							
sum y												101.68							
Sum (xi*yi)												832.53							
sum x												47.00							
sum (X^2)												979.00							



**Table 4.136** Test 8  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the non-ferrous based  $O_2$  scavenger treatment in Lane A

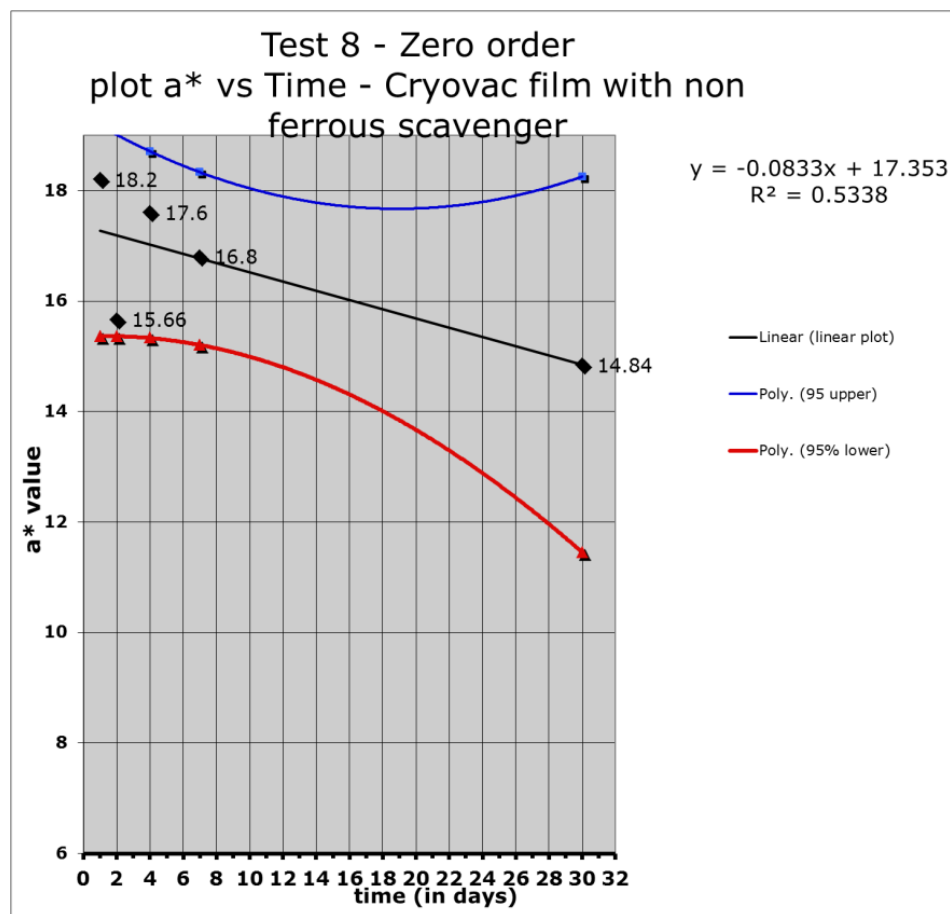
1. Raw Data:														
# data pairs Total=	6 This is automatically counted													
Y units	a'													
X units	days													



**Figure 4.87** lane A: Cryovac non –ferrous package Ham Zero order plot of  $a^*$  vs. time (30 days) with 95 % confidence limits calculation

**Table 4.137** Test 8  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the non-ferrous based  $O_2$  scavenger treatment in Lane B

1. Raw Data:															
# data pairs	Total=	5	This is automatically counted												
Y units	a'	Lane B non-ferrous													
X units	days														
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
18.2	1.0	331.24	18.20	17.27	1.00	18.20	17.27	0.87	60.84	18.20	1.00	19.17	15.37	3.81	17.27
15.66	2.0	245.24	15.66	17.19	4.00	15.66	17.19	2.33	46.24	31.32	4.00	19.01	15.36	3.65	17.19
17.6	4.0	309.76	17.60	17.02	16.00	17.60	17.02	0.34	23.04	70.40	16.00	18.71	15.33	3.38	17.02
16.8	7.0	282.24	16.80	16.77	49.00	16.80	16.77	0.00	3.24	117.60	49.00	18.33	15.21	3.13	16.77
14.84	30.0	220.23	14.84	14.85	900.00	14.84	14.85	0.00	449.44	445.20	900.00	18.25	11.45	6.80	14.85
		0.00	0.00	17.35	0.00	0.00	17.35	301.13	77.44	0.00	0.00	19.34	15.36	3.98	17.35
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
slope= -0.0833															
intercept= 17.3532															
rsq= 0.5338															
± 95% slope 0.1429															
k upper 0.0596															
k lower -0.2263															
Equations															
Y = 17.3532 -0.0833 * time															
Standard Error 1.09															
Sum (yi-yes) 1810.34															
n 5.00															
t 95%, 2, n-2= 3.18															
x average = 8.80															
Sum (xi-xav) 1047.44															
(Sum x)^2 1936.00															
Sum (y^2) 1388.70															
sum y 83.10															
Sum (xi*yi) 682.72															
sum x 44.00															
sum (X^2) 970.00															



**Figure 4.88** lane B: Cryovac non –ferrous package Ham Zero order plot of  $a^*$  vs. time (30 days) with 95 % confidence limits calculation

1. Raw Data:			
# data pairs Total=	5	This is automatically counted	
Y units	a*	Lane C non-ferrous	
X units	days		

STATISTICS															
2. Calculati: Note after entering Y and X you need to pull down formulas in each column from top to last entry r(yi-yes)*2															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%LL	Delta	predicte average
18.19	1.0	330.88	18.19	17.40	1.00	18.19	17.40	0.62	49.00	18.19	1.00	18.49	16.31	2.18	17.40
16.67	2.0	277.89	16.67	17.40	4.00	16.67	17.40	0.53	36.00	33.34	4.00	18.45	16.35	2.09	17.40
17.57	3.0	308.70	17.57	17.40	9.00	17.57	17.40	0.03	25.00	52.71	9.00	18.41	16.39	2.02	17.40
17.12	4.0	293.09	17.12	17.39	16.00	17.12	17.39	0.07	16.00	68.48	16.00	18.37	16.41	1.96	17.39
17.33	30.0	300.33	17.33	17.29	900.00	17.33	17.29	0.00	484.00	519.80	900.00	18.34	16.24	4.10	17.29
		0.00	0.00	17.41	0.00	0.00	17.41	303.03	64.00	0.00	0.00	18.54	16.27	2.27	17.41
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)*2	(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%LL	Delta	predicte average

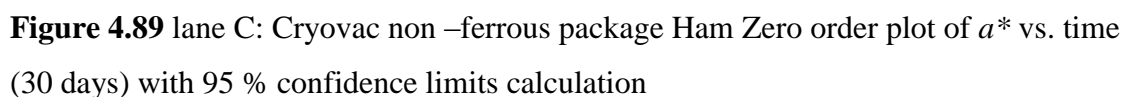
slope=	-0.0040
intercept=	17.4077
rsq=	0.0076
± 95% slope	0.0833
k upper	0.0794
k lower	-0.0873

Equations	
Y = 17.4077	-0.0040 * time

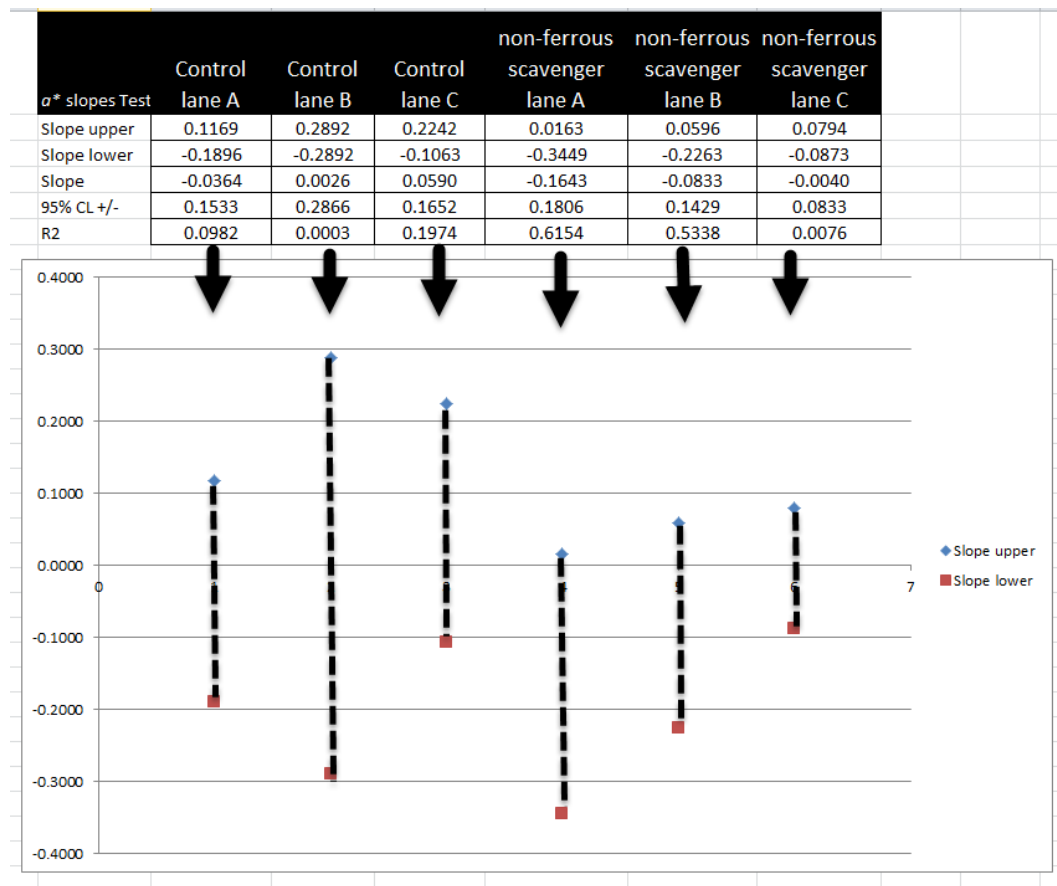
  

Standard Er	0.65
Sum (yi-yes)	1819.43
n	5.00
t 95%, 2, n-2=	3.18
x average =	8.00
Sum (xi-xav)	994.00
(Sum x)^2	1600.00
Sum(y^2)	1510.89
sum y	86.88
Sum (xi*yi)	692.62
sum x	40.00
sum (X^2)	930.00



Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments is provided in Table 4.139. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 4.139**  $a^*$  Rate constant ( $k$ ) upper and lower for the control package and non-ferrous package in test 8 lane A, B, and C as established by Labuza' Reaction kinetics shelf life model.



The largest range (i.e. more volatile and less predictable) of slope outcomes occurs in the control in lane B (+/- 28.66) (Table 4.139). The non-ferrous oxygen scavenger film was more likely than the control package to develop negative slopes over time; however the performance of this film is not statistically different from the control package.

The fit of data (as measured by  $R^2$ ) was again low for all treatments due to the limited number of samples and high variability in actual  $a^*$  color scores over time which is attributed to both the starting ham color variation (due to the formulation) and changes due to photo-oxidation during refrigerated shelf life. The trend line for each treatment shows negative slopes (lose of redness) for all lanes in the non-ferrous scavenger treatment, while the control package shows positive slopes for lanes B and C, and a negative line for lane A. In previous testing (Test 6), the control package in lane A and C also projected a negative trend line, with lane B being a positive. Though lane performance has not been demonstrated to be statistically different, lane A has often resulted in a larger rate constant at the 95% CL, suggesting greater variability in lane A, and more unpredictable results.

#### 4.8.5 – $L^*$ scores Test 8

The variability of  $L^*$  values across both treatments over time was large but similar to each other with a range of scores for the control across all lanes from 56.93 to 64.42 ( $\Delta L^* = 7.49$ ) and a range of 56.23 to 62.12 ( $\Delta L^* = 5.89$ ) for the non-ferrous scavenger (Table 4.140).

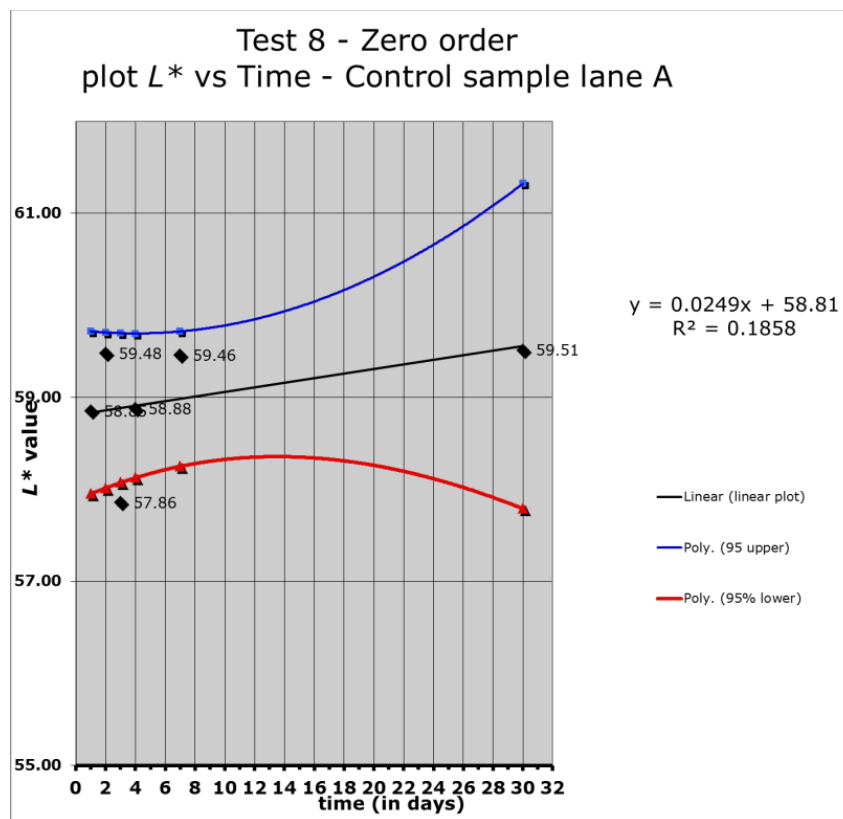
**Table 4.140**  $L^*$  scores for all treatments in all lanes (A, B, and C) Test 8

day	Control lane A $L^*$	Control lane B $L^*$	Control lane C $L^*$	Oxygen scavenging film lane A $L^*$	Oxygen scavenging film lane B $L^*$	Oxygen scavenging film lane C $L^*$
1	58.85	58.28	58.26	59.38	56.23	57.86
2	59.48	62.69	57.58	59.33	60.41	59.31
3	57.86	61.80	59.49	60.74	58.31	57.55
4	58.88	58.06	56.93	57.43	56.69	57.45
7	59.46	64.42	59.06	58.36	59.85	59.74
30	59.51	61.00	58.50	62.12	59.19	58.36
<b>min</b>	57.86	58.06	56.93	57.43	56.23	57.45
<b>max</b>	59.51	64.42	59.49	62.12	60.41	59.31
<b>range</b>	1.66	6.36	2.56	4.69	4.18	1.86

This range is similar to the Test 7 results where the ferrous based scavenger sachet range across all lanes was from 56.58 to 61.44 ( $\Delta L^* = 4.86$ ), UV film only from 55.93 to 62.82 ( $\Delta L^* = 6.89$ ), and the combined package at 57.22 to 65.24 ( $\Delta L^* = 8.02$ ). This is also similar to the range found in the control packages in Test 6 (57.36 to 65.47 ( $\Delta L^* = 8.11$ )). Similar to the outcome with  $a^*$  values, none of the previous tests have found this  $L^*$  range of difference to be statistically different from each other, but has demonstrated the some treatments result in a narrow range of outcomes that allow for greater predictability. Entering the  $L^*$  values from Table 4.140 above into the kinetics data input sheet (Tables 4.141 – 4.146) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey pigment or concentration of pigments (which is also an indication of moisture loss).

**Table 4.141** Test 8  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for control packaging sandwiches in Lane A

1. Raw Data:																
# data pairs Total=		6 This is automatically counted														
Y units	L*	Lane A control														
X units	days															
STATISTICS																
2. Calculati Note after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yes)^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average	
	58.85	1.0	3463.32	58.85	58.84	1.00	58.85	58.84	0.00	46.69	58.85	1.00	59.72	57.95	1.77	58.84
	59.48	2.0	3537.47	59.48	58.86	4.00	59.48	58.86	0.38	34.03	118.95	4.00	59.71	58.01	1.69	58.86
	57.86	3.0	3347.39	57.86	58.89	9.00	57.86	58.89	1.06	23.36	173.57	9.00	59.70	58.07	1.62	58.89
	58.88	4.0	3466.85	58.88	58.91	16.00	58.88	58.91	0.00	14.69	235.52	16.00	59.69	58.13	1.57	58.91
	59.46	7.0	3535.10	59.46	58.98	49.00	59.46	58.98	0.22	0.69	416.20	49.00	59.72	58.25	1.47	58.98
	59.51	30.0	3541.84	59.51	59.56	900.00	59.51	59.56	0.00	491.36	1785.40	900.00	61.32	57.79	3.53	59.56
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	Statistics															
	slope=											Standard Error		0.64		
	intercept=											Sum (yi-yes)		17294.90		
	rsq=											n		6.00		
	± 95% slope											t 95%,2,n-2=		2.78		
	k upper											x average =		7.83		
	k lower															
	Equations															
	Y = 58.8103 0.0249 * time															
												Sum (xi-xav		917.64		
												(Sum x)^2		2209.00		
												Sum(y^2)		20891.98		
												sum y		354.03		
												Sum (xi*yi)		2788.49		
												sum x		47.00		
												sum (X^2)		979.00		

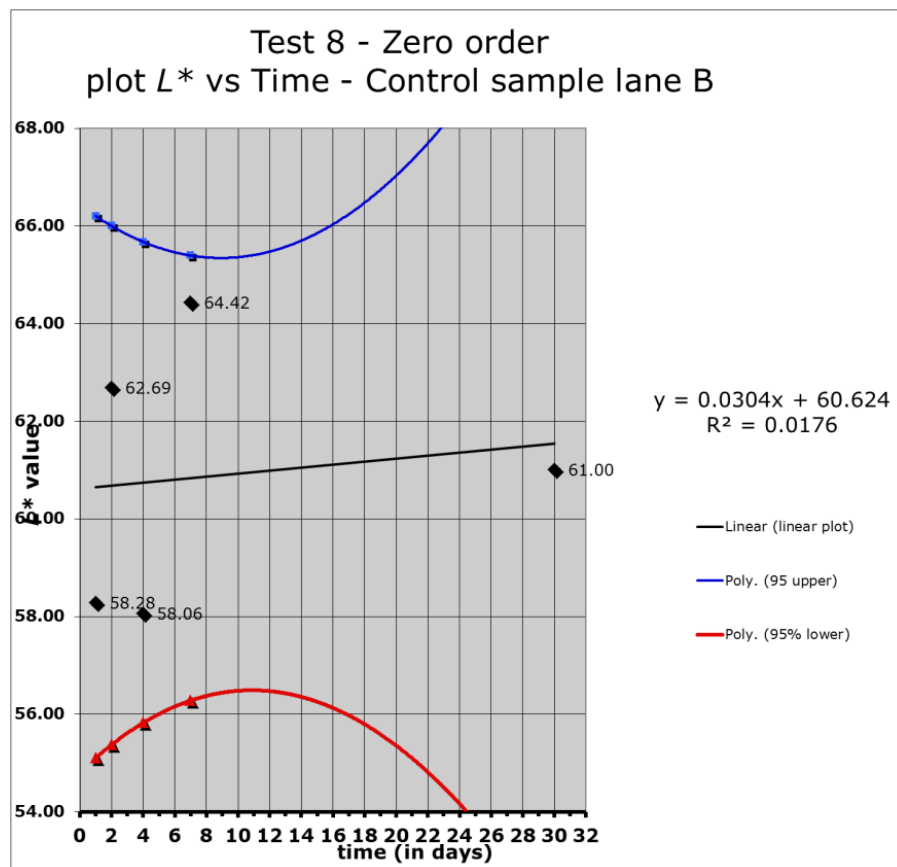


**Figure 4.90** lane A: control package Ham Zero order plot of  $L^*$  vs. time (30 days) with 95 % confidence limits calculation



**Table 4.142** Test 8  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for control packaging sandwiches in Lane B

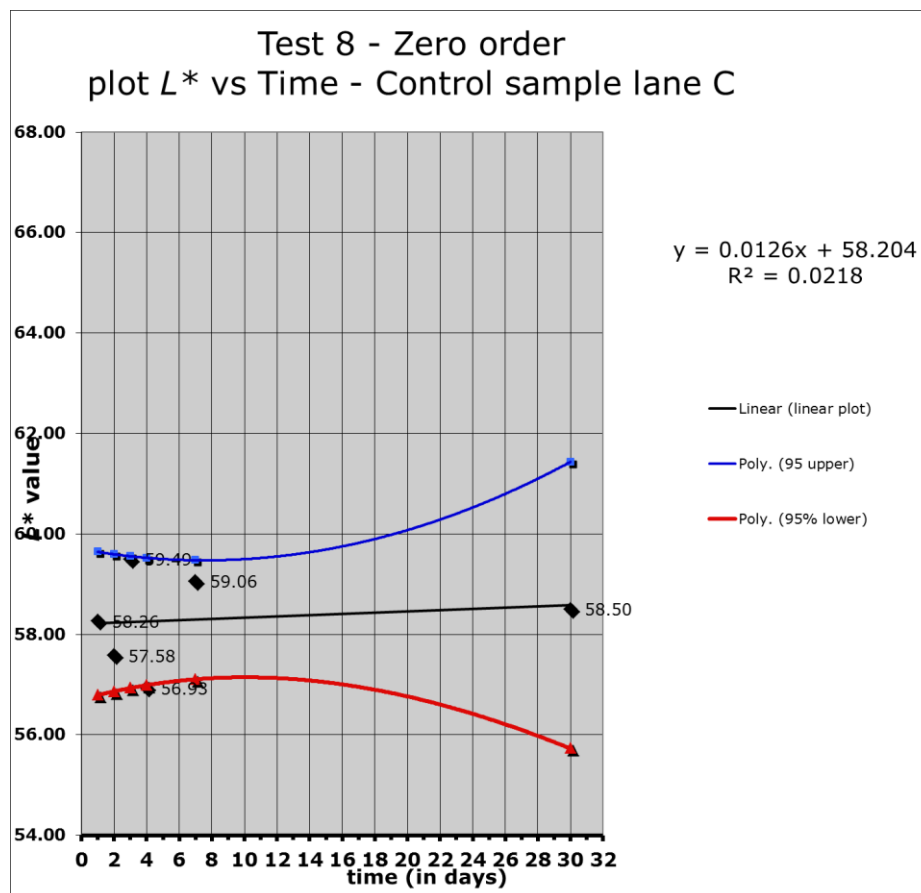
1. Raw Data:															
# data pairs Total=			5 This is automatically counted												
Y units	L*	Lane B	control												
X units	days														
STATISTICS															
2. Calculati Note after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yesy^2															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average	
58.28	1.0	3396.96	58.28	60.65	1.00	58.28	60.65	5.62	60.84	58.28	1.00	66.20	55.11	11.10	60.65
62.69	2.0	3930.04	62.69	60.69	4.00	62.69	60.69	4.02	46.24	125.38	4.00	66.00	55.37	10.63	60.69
58.06	4.0	3370.96	58.06	60.75	16.00	58.06	60.75	7.21	23.04	232.24	16.00	65.67	55.82	9.84	60.75
64.42	7.0	4150.37	64.42	60.84	49.00	64.42	60.84	12.86	3.24	450.96	49.00	65.40	56.28	9.12	60.84
61.00	30.0	3721.41	61.00	61.54	900.00	61.00	61.54	0.28	449.44	1830.10	900.00	71.45	51.63	19.82	61.54
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average



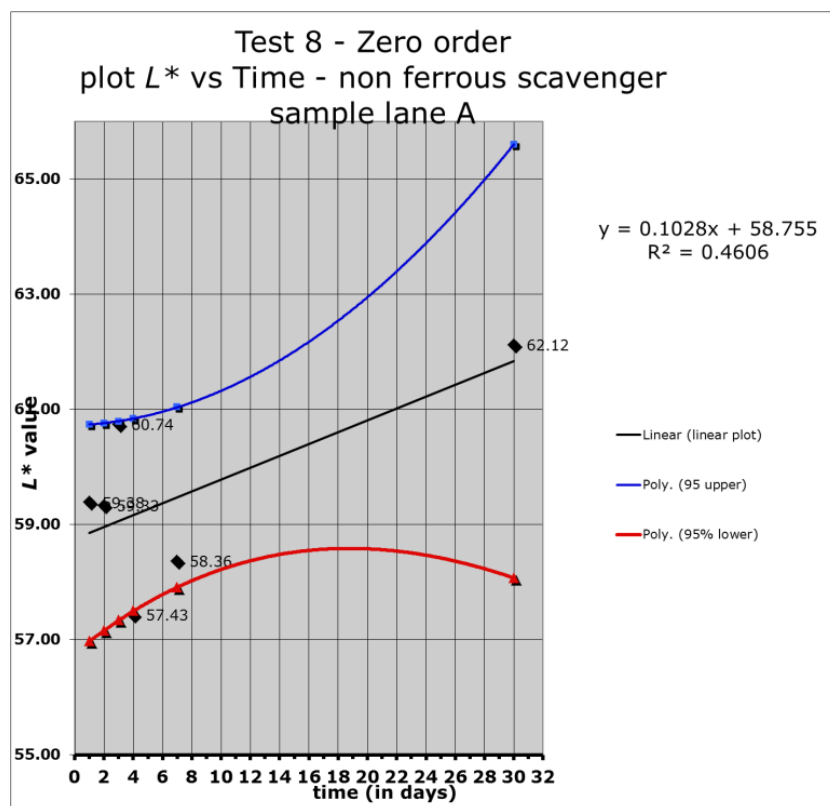
**Figure 4.91** lane B: control package Ham Zero order plot of  $L^*$  vs. time (30 days) with 95 % confidence limits calculation

**Table 4.143** Test 8  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for control packaging sandwiches in Lane C

1. Raw Data:														
# data pairs Total=	6	This is automatically counted												
Y units	L*	Lane C	control											
X units	days													
STATISTICS														
2. Calculati Note after entering Y and X you need to pull down formulas in each column from top to last entry rdyi-yesy^2														
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
58.26	1.0	3394.62	58.26	58.22	1.00	58.26	58.22	0.00	46.69	58.26	1.00	59.64	56.79	2.86
57.58	2.0	3315.07	57.58	58.23	4.00	57.58	58.23	0.43	34.03	115.15	4.00	59.59	56.86	2.73
59.49	3.0	3539.46	59.49	58.24	9.00	59.49	58.24	1.57	23.36	178.48	9.00	59.55	56.93	2.62
56.93	4.0	3241.02	56.93	58.25	16.00	56.93	58.25	1.75	14.69	227.72	16.00	59.52	56.99	2.53
59.06	7.0	3487.69	59.06	58.29	49.00	59.06	58.29	0.58	0.69	413.40	49.00	59.48	57.11	2.37
58.50	30.0	3421.86	58.50	58.58	900.00	58.50	58.58	0.01	491.36	1754.90	900.00	61.44	55.73	5.71
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta
												Standard Error	1.04	
slope=												Sum (yi-yes)	16943.02	
intercept=												n	6.00	
rsq=												t 95%,2,n-2=	2.78	
± 95% slope												x average =	7.83	
k upper												Sum (xi-xav)	917.64	
k lower												(Sum x)^2	2209.00	
												Sum(y^2)	20399.72	
Equations												sum y	349.82	
Y = 58.2043 + 0.0126 * time												Sum (xi*yi)	2747.91	
												sum x	47.00	
												sum (X^2)	979.00	



**Figure 4.92** lane C: control package Ham Zero order plot of  $L^*$  vs. time (30 days) with 95 % confidence limits calculation

[illegible]

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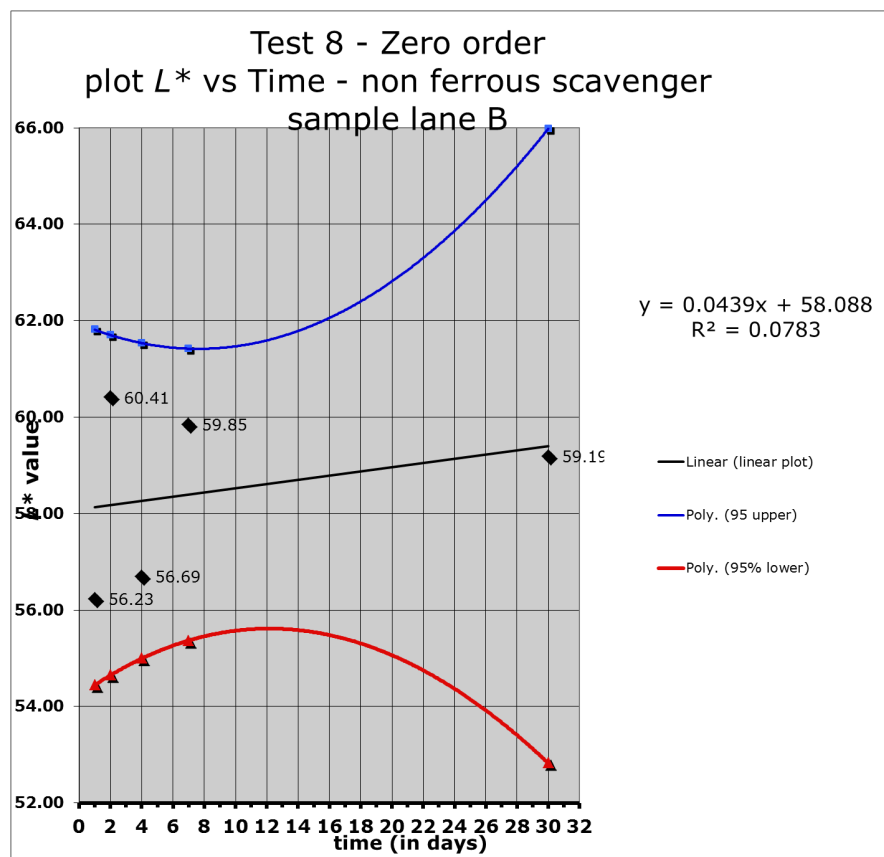
1. Raw Data:		
# data pairs Total=	5	This is automatically counted
Y units	L*	Lane B
X units	days	non-ferrous scavenger

STATISTICS															
2. Calculati-Note after entering Y and X you need to pull down formulas in each column from top to last entry (y1-yes)*2															
Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	yi estimate	(y1-yes)*2	(xi-xave)^2	Xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
56.23	1.0	3162.19	56.23	58.13	1.00	56.23	58.13	3.60	60.84	56.23	1.00	61.82	54.45	7.37	58.13
60.41	2.0	3649.37	60.41	58.18	4.00	60.41	58.18	4.99	46.24	120.82	4.00	61.70	54.65	7.06	58.18
56.69	4.0	3213.76	56.69	58.26	16.00	56.69	58.26	2.48	23.04	226.76	16.00	61.53	55.00	6.54	58.26
59.85	7.0	3581.62	59.85	58.39	49.00	59.85	58.39	2.11	3.24	418.93	49.00	61.42	55.37	6.05	58.39
59.19	30.0	3503.46	59.19	59.40	900.00	59.19	59.40	0.05	449.44	1775.70	900.00	65.98	52.82	13.16	59.40
		0.00	0.00	58.09	0.00	0.00	58.09	3374.18	77.44	0.00	0.00	61.94	54.24	7.70	58.09
Y value	x=time	Y^2	Y plot value	Est y1	time^2	y1	yi estimate	(y1-yes)*2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
slope=												0.0439			
intercept=												58.0877			
rsq=												0.0783			
± 95% slope												0.2766			
k upper												0.3205			
k lower												-0.2327			
Equations															
Y = 58.0877 + 0.0439 * time															

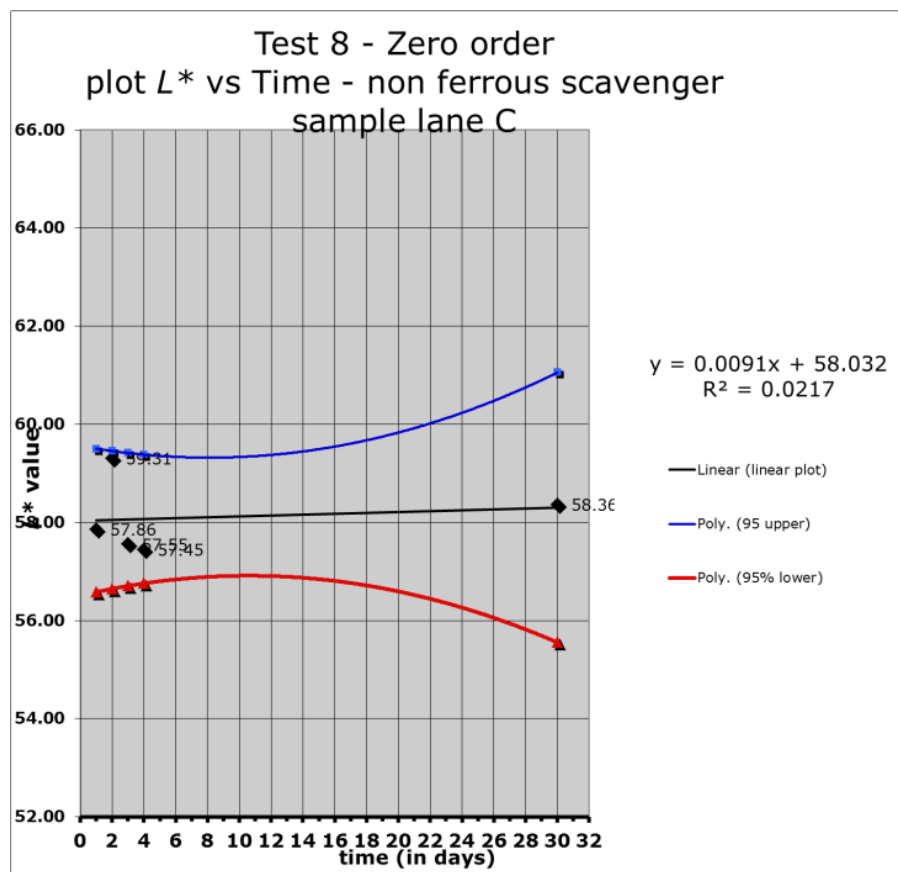
Standard Error	2.10
Sum (y1-yes)	20258.31
n	5.00
t 95%,2,n-2=	3.18
x average =	8.80
Sum (xi-xav)	1047.44
(Sum x)^2	1936.00
Sum(y^2)	17110.39
sum y	292.37
Sum (xi^*yi)	2598.44
sum x	44.00
sum (X^2)	970.00



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**Table 4.146** Test 8  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for non-ferrous scavenging packaging sandwiches in Lane C

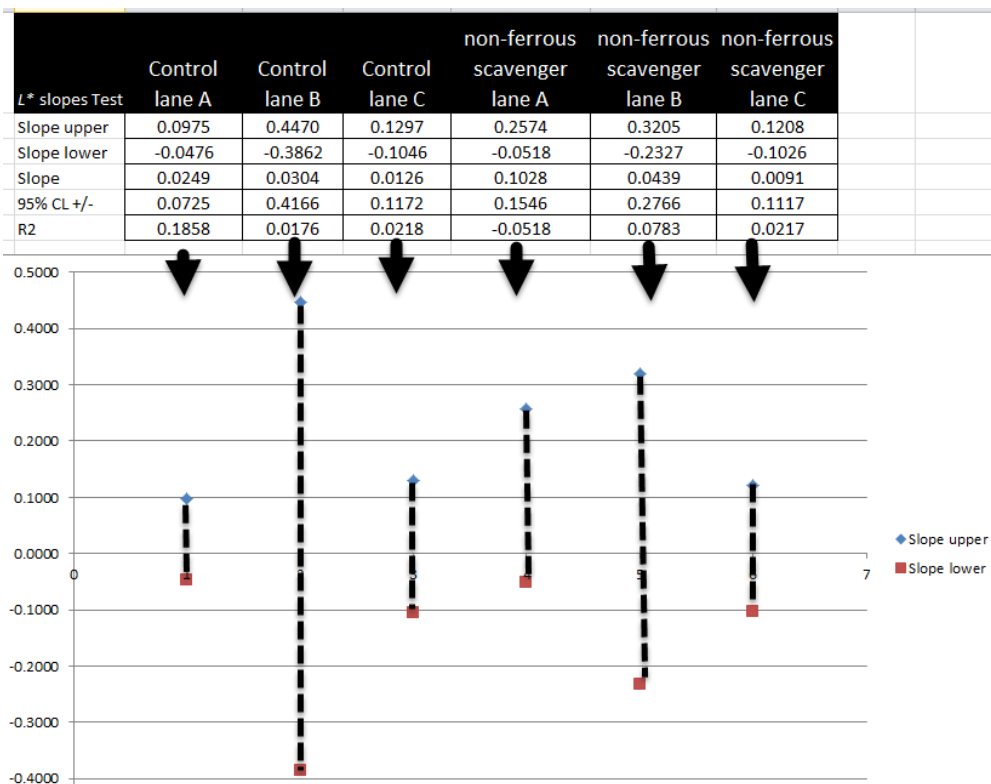
1. Raw Data:															
# data pairs		Total=	5	This is automatically counted											
Y units	L*	Lane C	non-ferrous scavenger												
X units	days														
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry row (y1-yes)*2															
Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	y1 estimate	(y1-yes)^2	(xi-xave)^2	xi*y1	X^2	y 95%UL	y 95%LL	Delta	predicte average
57.86	1.0	3347.39	57.86	58.04	1.00	57.86	58.04	0.03	49.00	57.86	1.00	59.50	56.58	2.92	58.04
59.31	2.0	3517.28	59.31	58.05	4.00	59.31	58.05	1.58	36.00	118.61	4.00	59.45	56.65	2.81	58.05
57.55	3.0	3312.39	57.55	58.06	9.00	57.55	58.06	0.26	25.00	172.66	9.00	59.41	56.70	2.71	58.06
57.45	4.0	3300.12	57.45	58.07	16.00	57.45	58.07	0.39	16.00	229.79	16.00	59.38	56.76	2.62	58.07
58.36	30.0	3405.89	58.36	58.30	900.00	58.36	58.30	0.00	484.00	1750.80	900.00	61.05	55.55	5.50	58.30
		0.00	0.00	58.03	0.00	0.00	58.03	3367.73	64.00	0.00	0.00	59.56	56.51	3.05	58.03
Y value	x=time	Y^2	Y plot value	Est y1	time^2	y1	y1 estimate	(y1-yes)^2	(xi-xave)^2	xi*y1	X^2	y 95%UL	y 95%LL	Delta	predicte average
slope=												Standard Error			
0.0091												0.87			
intercept=												Sum (y1-yes)			
58.0321												20208.64			
rsq=												n			
0.0217												5.00			
± 95% slope												t 95%, 2, n-2=			
0.1117												3.18			
k upper												x average =			
0.1208												8.00			
k lower															
-0.1026												Sum (xi-xav)			
												994.00			
												(Sum x)^2			
												1600.00			
												Sum (y^2)			
												16883.07			
												sum y			
												290.52			
												Sum (xi*y1)			
												2329.72			
												sum x			
												40.00			
												sum (X^2)			
												930.00			
Equations															
Y = 58.0321 0.0091 * time															



**Figure 4.95** lane C: non-ferrous scavenger package Ham Zero order plot of  $L^*$  vs. time (30 days) with 95 % confidence limits calculation

Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges ( $+k$  for lightening over the shelf life,  $-k$  for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.147.

**Table 4.147**  $L^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in test 8 as established by Labuza' Reaction kinetics shelf life model



Both treatments demonstrated a larger range in variability at the 95% confidence level in lane B (control slope  $\pm$  0.4166, non-ferrous scavenger slope  $\pm$  0.2766) (Table 4.147). The non-ferrous scavenger treatment was more likely to develop positive slopes over time (indicating a lightening or fading of the product). However, there is overlap of potential outcomes for all treatments which makes the performance for each treatment not statistically different.

#### **4.8.6 - Visual appearance of the ham Test 8**

Both the control and non-ferrous based scavenger film developed visual discoloration within 2 days of refrigerated shelf life (Appendix H.2). With oxygen present in the headspace through day 4 in both packages, development of visual discoloration was expected. Visual discoloration and O<sub>2</sub> levels with both treatments proceeding to 0% by day 7 in lane A, and 0% O<sub>2</sub> in all lanes by day 30, suggest that part of the oxygen consumption in the package is attributed to metmyoglobin formation.

#### **4.8.7 – Cooler temperatures Test 8**

The coolers utilized were the same coolers used in Tests 2-7. The settings were not adjusted. Temperature was not tracked in this study. In Tests 2-7, the coolers established a consistent average temperature between 0.0 – 1.0 C°.

#### **4.8.8 – Conclusions Test 8**

This test confirms the speculation that the non-ferrous based oxygen scavenging film does not work at freezing temperatures (Optimal performing temperature range is 3.3 – 21°C. (Cryovac<sup>®</sup>)). Cryovac cautioned that once the UV / free radical reaction is terminated, it cannot be re-initiated upon thawing. This is because the scavenging reaction is initiated when free radicals are generated from UV energy. (See section 3.7) The scavenging reaction (formation of the free radicals) usually peaks in 24 to 48 hours, but can be even longer because of reduced access of oxygen to the film in a low oxygen MAP package (because the scavenging mechanism in both the top and bottom film is located between film layers). The risk of a long freezing period is that the free radicals are extinguished before the reaction can gain momentum. (S. Beckwith, personal communication, February 25, 2015) The benefit of exploring this option was it offered a solution that wasn't a challenge with current metal detection and for all practical purposes was a clear film that didn't obscure the sandwich appearance. This film may be viable for a process that has MAP followed by dark refrigeration only, or for an extended period of refrigeration before frozen storage. Current constraints at the sandwich manufacturing facility does not allow for this potential, so no further exploration will be

done on non-ferrous based oxygen scavenging film. This remains a consideration for future research if short term dark, refrigerated storage is available. A key element of a future study would be to establish what length of time for dark refrigerated storage is required to achieve 0% O<sub>2</sub> prior to sandwich freezing or display. Dark refrigerated storage is essential for future tests. Photo-oxidation occurs when light absorption of heme protein causes nitrosylmyoglobin to dissociate into nitric oxide and myoglobin (Johnston, Knight and Ledward, 1992). When this happens in the presence of O<sub>2</sub>, formation of metmyoglobin can be thermodynamically favorable over other potential reactions.



## **4.9 - Test 9 ferrous based oxygen scavenging film, revisit oxygen scavenging sachet and non-ferrous based scavenging film**

### **4.9.1 – Test 9 overview**

The goal of this test was to compare the performance of a 1) ferrous based oxygen scavenging film (Winpak®); 2) ferrous based oxygen scavenging sachet (Multisorb® D-50); 3) non-ferrous based oxygen scavenging film (Cryovac®) with one day storage in refrigeration prior to freezing; and 4) the current MAP package (control).


In this study, a new ferrous based oxygen scavenging film is evaluated. Winpak® combines both passive and active barrier technologies in a polyester lamination with a high barrier EVOH and linear low density polyethylene co-extrusion sealant with scavenging additive. While the passive barrier EVOH is designed to stop the ingress of oxygen, the active barrier removes intra-package oxygen using a chemical absorption process. The chemical absorption process converts the ferrous iron powder embedded in the sealant film with free oxygen in the package into a stable ferric oxide. In this system there is no generation of byproducts that may affect the organoleptic properties of the food (R.Klips, personal communication, August 10, 2015). The packaging appearance is translucent, with a gray tint. The grey tint is noticeable and will require consumer validation for an acceptable appearance if it is found to enhance the color performance of the ham. The film is produced by Winpak® Ltd. in Manitoba, Canada.

Based on the findings that freezing the film immediately following packaging terminated the scavenging reaction in test 8, the Cryovac® non-ferrous O<sub>2</sub> scavenging film was revisited to see if 24 hours of refrigerated dark storage (approximately 3.3°C) prior to freezing provided enough time to effectively remove oxygen before freezing. The optimal performing temperature range for the Cryovac® non-ferrous O<sub>2</sub> scavenging film is 3.3 – 21°C.

The Multisorb® D-50 scavenging sachet is evaluated for repeatability of results from Test 7.

### 4.9.2 – Methods and Materials Test 9

Four Beverage Air cooler (Model # LV27 c) with fluorescent bulbs were used in this study (the coolers designated “A”, “B” and “C” from previous studies. Another identical cooler “D” was added). Each cooler set contained only one test variable, with vertical lanes A and B loaded one sandwich deep on the front lip (Figure 4.96).

Meat Discoloration Study 9			
	lane B	lane A	
Cooler A	2	1	Example of cooler set
Test - Cryovac	4	3	
	6	5	
	8	7	
	10	9	
Cooler B	2	1	
Test - Winpak	4	3	
	6	5	
	8	7	
	10	9	
Cooler C	2	1	
Control Current Film	4	3	
	6	5	
	8	7	
	10	9	
Cooler D	2	1	
Test - Scavenger	4	3	
	6	5	
	8	7	
	10	9	

**Figure 4.96** Cooler set up configuration for Test 9. Five shelves were utilized for a total of 10 sandwiches per cooler. The color coding is by lane. Light blue represents lane A, and light green tinted represents lane B.

Sandwiches were removed on each designated day and evaluated five times throughout a 7 day refrigerated shelf life for oxygen percentage in the package headspace, Ham  $L^*$  and  $a^*$  color analysis (removed from the package) and visual evaluation (photographs documented in Appendix I). A summary of the sample numbers evaluated and corresponding day in shelf life are listed in Table 4.148.

**Table 4.148** Test 9 sample numbers evaluated and corresponding day in shelf life

Date	Day	Cryovac	Winpak	Control	Multisorb
		Cooler A	Cooler B	Cooler C	Cooler D
		Control sample	sample	sample	sample
		numbers	numbers	numbers	numbers
		evaluated	evaluated	evaluated	evaluated
3/4/2014	sandwiches assembled, packaged and frozen				
4/14/2014	refrigerated shelf life begins				
4/15/2014	1	1, 2	1, 2	1, 2	1, 2
4/16/2014	2	3, 4	3, 4	3, 4	3, 4
4/17/2014	3	5, 6	5, 6	5, 6	5, 6
4/18/2014	4	7, 8	7, 8	7, 8	7, 8
4/21/2014	7	9, 10	9, 10	9, 10	9, 10

The ham, cheese, and bread utilized were consistent with tests 1-8 and are described in methods and materials section 3.1. Each sandwich component used was from the same production lot to minimize batch to batch variability. The ham and cheese was stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly. The bread was stored at room temperature (approximately 21° C) prior to assembly. The length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The age of the ham at the time of packaging was 26 days old.

The control packaging used is as described in section 3.3 (Belmark clear non-forming film with O<sub>2</sub> barrier) and 3.4 (Curwood clear forming film with O<sub>2</sub> barrier). For the test packaging, the Multisorb D-50 sachet used is as described in Test 7, and is combined with the control packaging. Cryovac® top (non-forming) film and bottom forming film is used as described in Test 8. In this study, a new ferrous based oxygen scavenging film is considered. The Winpak® ferrous based film is a polyester lamination with a high barrier EVOH and linear low density polyethylene co-extrusion sealant with additive (Winpak®). It does not require any special equipment to run on a packaging line. Moisture is the only component required to activate scavenging. For this reason, rolls stored in high humidity are recommended to be wrapped in film capable of blocking moisture from activating the scavenging process prematurely. The oxygen absorption capacity of Winpak®'s oxygen scavenger film is dependent on three variables: 1) size of the package, 2) quantity of oxygen scavenger additive, 3) relative humidity inside the package. The theoretical capacity of the additive is 100cc O<sub>2</sub> per gram of additive.

However, when the relative humidity is below 100%, this capacity is reduced. The capacity of the additive reduces to zero at relative humidity levels below 40%. Winpak® recommends a 70% target level of humidity to maximize benefits from the oxygen scavenger. The oxygen scavenger film was designed for use at refrigerated and at room temperatures. The amount of ferrous iron powder in the sealant layer can be adjusted. The maximum theoretical capacity of the oxygen scavenger film is 1cc O<sub>2</sub>/in<sup>2</sup>. For this test 0.3cc O<sub>2</sub>/in<sup>2</sup> was utilized. If effective, the cost of the scavenger film solution is approximately 2.5 times the typical MAP barrier film with EVOH.

All sandwiches were assembled and placed in MAP (Modified Atmosphere Packaging) with an 80% N<sub>2</sub> / 20% CO<sub>2</sub> blend (Materials and Methods section 3.15) at E.A. Sween Company using a Multivac R530 (Materials and Methods section 3.19). All sandwiches were produced within nine cycles of the machine (approximately 60 seconds per cycle). The packaging configuration used is the flat faced square format package (1 to 1.8 product to package ratio). While the control and other test applications were placed in frozen storage (0°C) immediately after assembly and packaging, the non-ferrous based film samples were placed in refrigerated storage (3.3°C) for 24 hours based on the supplier's recommendations. The sandwiches were all not labeled to maximize surface area of the ham exposed to light. All packaged sandwiches were stored in dark frozen storage in a corrugated case for 27 days before the start of the refrigerated shelf life.

#### **4.9.3 – Oxygen percentages per package Test 9**

A summary of the percent O<sub>2</sub> in the headspace of all treatments during refrigerated shelf life is provided in Table 4.149. Carbon Dioxide values can be found in the Appendix I.6.

**Table 4.149** Oxygen percentages in the headspace for control and test film over time (all lanes) test 9. Red indicates packages identified as leakers.

	Control	Control	Winpak	Winpak	Cryovac	Cryovac	Multisorb	Multisorb
	Control lane	lane B O2	lane A	lane B	lane A	lane B	lane A O2	lane B O2
day	A O2 %	%	O2 %	O2 %	O2 %	O2 %	%	%
1	0.25	0.42	0.00	0.04	0.09	18.40	0.00	0.00
2	0.26	0.15	0.00	0.00	0.09	0.14	0.00	0.00
3	0.28	0.27	0.01	0.12	0.12	0.23	0.09	0.09
4	0.30	16.60	0.00	0.25	0.02	0.07	0.00	0.00
7	0.19	0.09	0.00	0.00	0.03	0.00	0.00	0.00
min	0.19	0.09	0.00	0.00	0.02	0.00	0.00	0.00
max	0.30	16.60	0.01	0.25	0.12	18.40	0.09	0.09
range	0.12	16.51	0.01	0.25	0.10	18.40	0.09	0.09

For the control, a range of 0.19% - 0.30% oxygen was achieved (Table 4.146). For the scavenger applications, the desired 0% oxygen on day 1 of shelf life was achieved with the Multisorb sachet and the lane A Winpak® sample. However at some point in the shelf life, all scavenging applications had residual oxygen present. For the sachet, the outcome was similar to the results in test 4 where 2 out of 20 packages using D-30 had a small amount of residual O<sub>2</sub> over time and reinforces the potential that proper circulation around the sachet is not occurring in all samples. Based on the result, 24 hours in dark refrigeration for the non-ferrous based scavenger prior to freezing was not effective in improving the residual O<sub>2</sub> outcome.

#### 4.9.4 – Ham *a*\* scores Test 9

The focus of this *a*\* analysis was to compare week 1 performance of all treatments in lanes A and B. The measured *a*\* values for all treatments is listed in Table 4.150. The leaker package *a*\* values are reported below (Table 4.150 in red), but not included in the minimum, maximum or statistical calculations.

**Table 4.150**  $a^*$  color scores both test and control film Test 9. Values in red indicate packages that were identified as leakers (improper seal) with high oxygen in the headspace

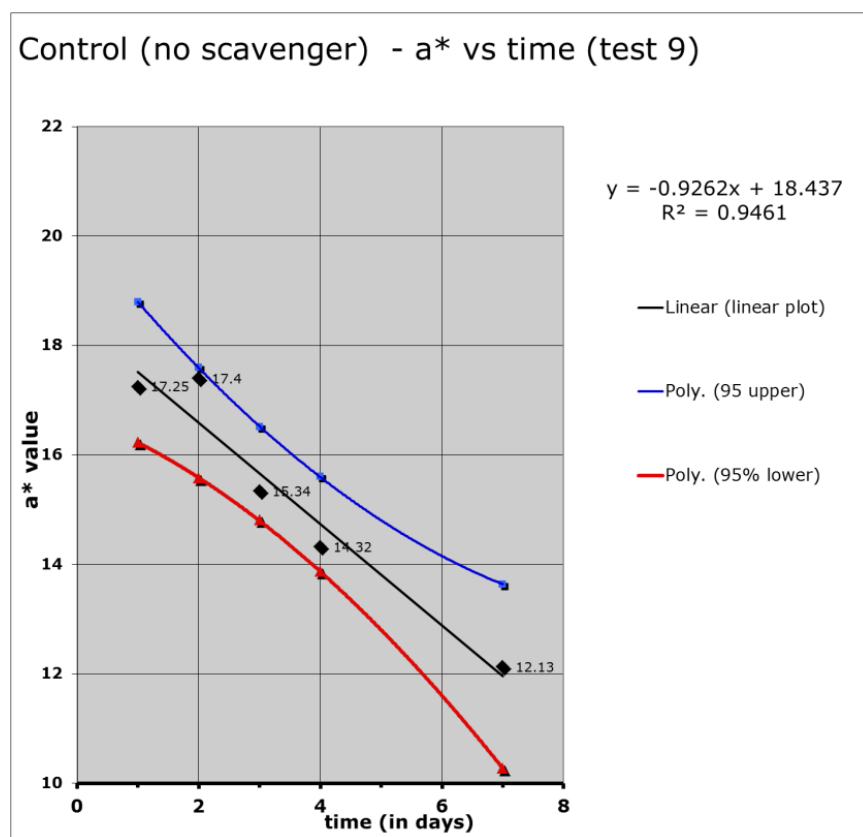
day	Control lane A $a^*$	Control lane B $a^*$	Winpak lane A $a^*$	Winpak lane B $a^*$	Cryovac lane A $a^*$	Cryovac lane B $a^*$	Multisorb lane A $a^*$	Multisorb lane B $a^*$
1	17.25	18.84	17.46	20.22	16.22	12.65	18.39	17.94
2	17.40	16.80	17.33	14.79	14.74	16.55	15.85	16.24
3	15.34	14.67	16.62	17.47	12.37	17.00	18.31	14.89
4	14.32	7.27	16.06	18.03	13.04	16.86	17.57	18.45
7	12.13	13.15	17.24	16.53	9.82	16.23	18.52	16.66
min	12.13	13.15	16.06	14.79	9.82	16.23	15.85	14.89
max	17.40	18.84	17.46	20.22	16.22	17.00	18.52	18.45
range	5.27	5.69	1.40	5.43	6.40	0.77	2.67	3.56

The  $\Delta a^*$  (maximum – minimum) was greatest in the control film samples (5.27, 5.69). The  $\Delta a^*$  was less for the Multisorb treatment (2.67, 3.56), and similar to the  $\Delta a^*$  observed in test 7 (1.83, 2.57, 1.53 for lanes A-C in test 7). The  $\Delta a^*$  was inconsistent between lanes A and B for the Winpak® (1.4, 5.43) and Cryovac treatments (6.4, 0.77). In test 8, the Cryovac film had a similar result with  $\Delta a^*$  =6.23 (lane A), 3.36 (lane B), and 1.52 (Lane C).

Entering the  $a^*$  values from Table 4.150 above into the kinetics data input sheet (Tables 4.151 – 4.158) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

**Table 4.151** Test 9  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the control package in Lane A

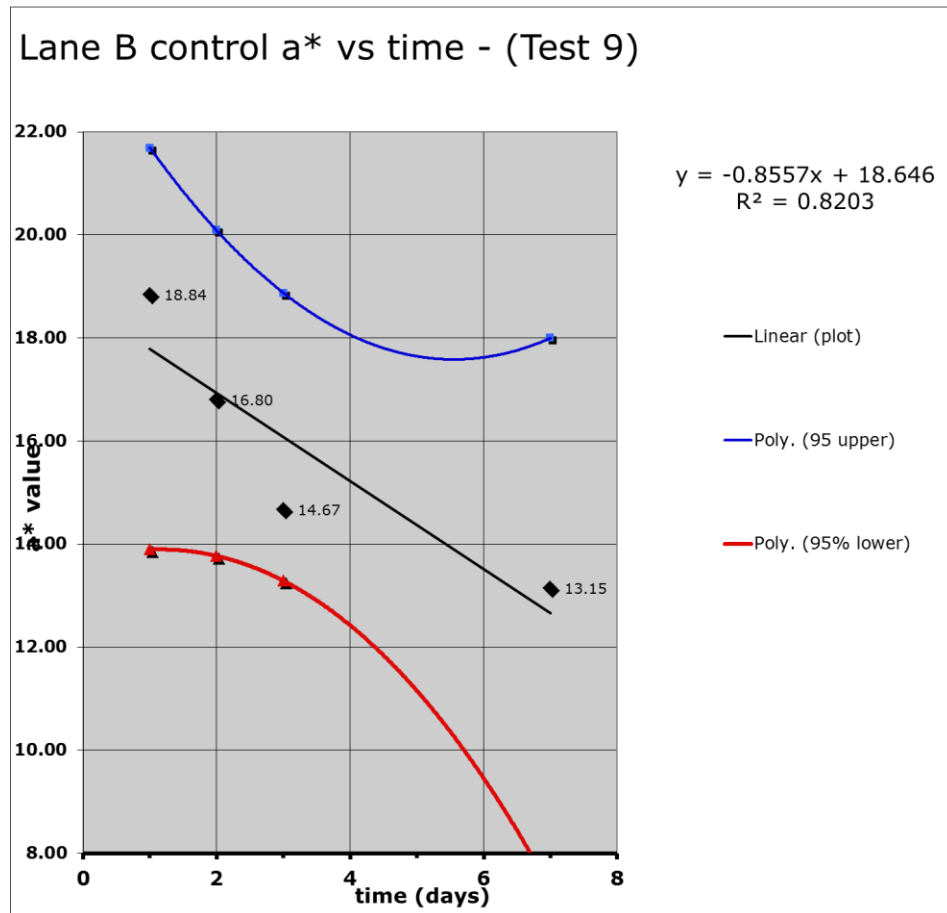
1. Raw Data:															
# data pairs	Total=	5	This is automatically counted												
Y units	a'														
X units	days														
2. Calculations:															
Note after entering Y and X you need to pull down formulas in each column from top to last entry $r(yi-yes)^2$															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
17.25	1.0	297.56	17.25	17.51	1.00	17.25	17.51	0.07	5.76	17.25	1.00	18.79	16.23	2.57	17.51
17.4	2.0	302.76	17.40	16.58	4.00	17.40	16.58	0.66	1.96	34.80	4.00	17.59	15.57	2.02	16.58
15.34	3.0	235.32	15.34	15.66	9.00	15.34	15.66	0.10	0.16	46.02	9.00	16.51	14.81	1.70	15.66
14.32	4.0	205.06	14.32	14.73	16.00	14.32	14.73	0.17	0.36	57.28	16.00	15.60	13.86	1.74	14.73
12.13	7.0	147.14	12.13	11.95	49.00	12.13	11.95	0.03	12.96	84.91	49.00	13.64	10.27	3.37	11.95
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predict average
STATISTICS															
												Standard Error	0.59		
												Sum (yi-yes)	2040.61		
												n	5.00		
												t 95%, 2, n-2=	3.18		
												x average =	3.40		
												Sum (xi-xav)	90.56		
												(Sum x)^2	289.00		
												Sum(y^2)	1187.84		
												sum y	76.44		
												Sum (xi*yi)	240.26		
												sum x	17.00		
												sum (X^2)	79.00		
Equations															
Y = 18.4372 - 0.9262 * time															
slope= -0.9262															
intercept= 18.4372															
rsq= 0.9461															
± 95% slope 0.4057															
k upper -0.5205															
k lower -1.3320															



**Figure 4.97** Lane A: Test 9 control Ham Zero order plot of  $a^*$  vs. time (7 days) with 95 % confidence limits calculation

**Table 4.152** Test 9  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the control package in Lane B

1. Raw Data:																																		
# data pairs Total=		4		This is automatically counted																														
Y units		a'		Lane B Control																														
X units		days																																
2. Calculations																																		
Note after entering Y and X you need to pull down formulas in each column from top to last entry																																		
STATISTICS																																		
2. Calculate: Note after entering Y and X you need to pull down formulas in each column from top to last entry																																		
Y value		x= time		Y^2		Y plot value		Est yi		time^2		yi		yi estimate		(yi-yes)^2		(xi-xave)^2		xi*yi		X^2		y 95%UL		y 95%LL		Delta		predicted average				
18.84		1.0		354.82		18.84		17.79		1.00		18.84		17.79		1.10		5.06		18.84		1.00		21.69		13.89		7.80		17.79				
16.80		2.0		282.35		16.80		16.93		4.00		16.80		16.93		0.02		1.56		33.61		4.00		20.10		13.77		6.33		16.93				
14.67		3.0		215.31		14.67		16.08		9.00		14.67		16.08		1.98		0.06		44.02		9.00		18.87		13.29		5.58		16.08				
13.15		7.0		172.83		13.15		12.66		49.00		13.15		12.66		0.24		14.06		92.03		49.00		18.00		7.31		10.69		12.66				
Y value		x= time		Y^2		Y plot value		Est yi		time^2		yi		yi estimate		(yi-yes)^2		(xi-xave)^2		xi*yi		X^2		y 95%UL		y 95%LL		Delta		predicted average				
															Statistics																			
slope=															-0.8557																			
intercept=															18.6459																			
rsq=															0.8203																			
± 95% slope															1.2178																			
k upper															0.3621																			
k lower															-2.0734																			
Equations																																		
Y = 18.6459															-0.8557 * time																			
															Standard Error										1.29									
															Sum (yi-yes)										2437.02									
															n										4.00									
															t 95%, 2, n-2										4.30									
															x average =										3.25									
															Sum (xi-xav)										94.69									
															(Sum x)^2										169.00									
															Sum(y^2)										1025.31									
															sum y										63.46									
															Sum (xi*yi)										188.49									
															sum x										13.00									
															sum (X^2)										63.00									

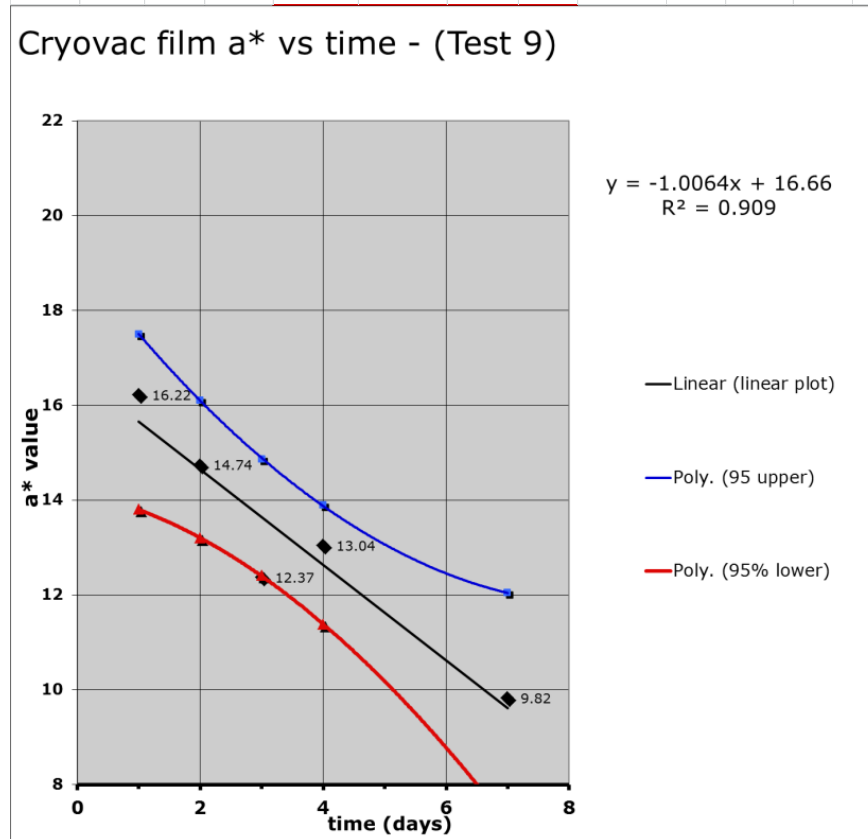


**Figure 4.98** Lane B: Test 9 control Ham Zero order plot of  $a^*$  vs. time (7 days) with 95 % confidence limits calculation

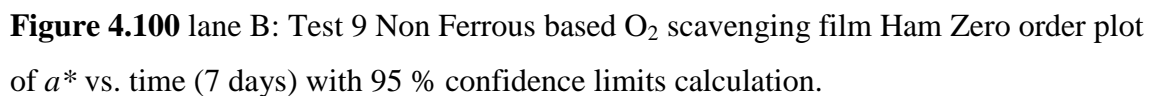


**Table 4.153** Test 9  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the Cryovac package in Lane A

1. Raw Data:															
# data pairs	Total=	5	This is automatically counted												
Y units	a'														
X units	days														
STATISTICS															
2. Calculati-Note after entering Y and X you need to pull down formulas in each column from top to last entry (y1-yes)^2															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
16.22	1.0	263.09	16.22	15.65	1.00	16.22	15.65	0.32	5.76	16.22	1.00	17.50	13.80	3.70	15.65
14.74	2.0	217.27	14.74	14.65	4.00	14.74	14.65	0.01	1.96	29.48	4.00	16.10	13.19	2.91	14.65
12.37	3.0	153.02	12.37	13.64	9.00	12.37	13.64	1.61	0.16	37.11	9.00	14.87	12.41	2.45	13.64
13.04	4.0	170.04	13.04	12.63	16.00	13.04	12.63	0.16	0.36	52.16	16.00	13.89	11.38	2.51	12.63
9.82	7.0	96.43	9.82	9.61	49.00	9.82	9.61	0.04	12.96	68.74	49.00	12.04	7.19	4.85	9.61
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average

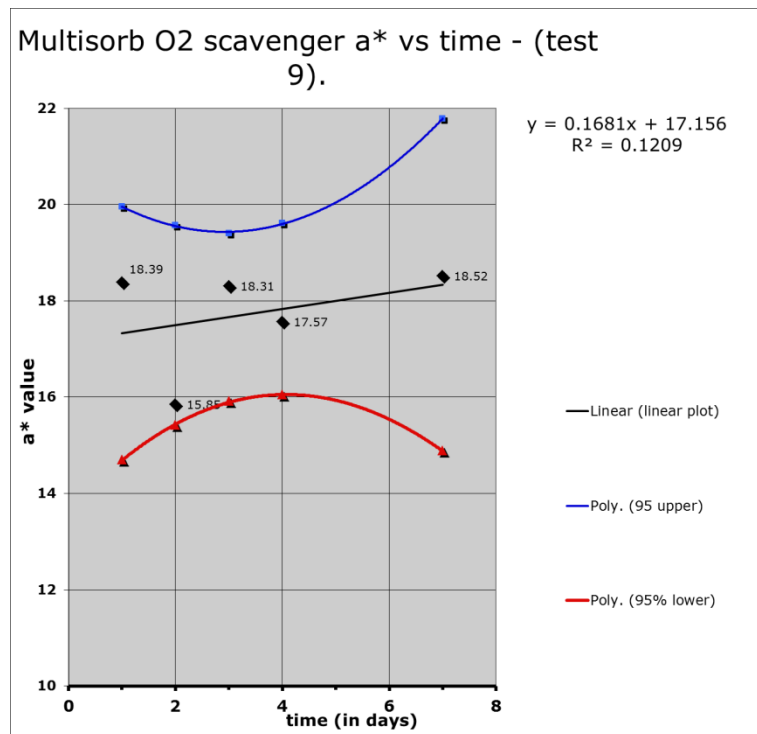


**Figure 4.99** lane A: Test 9 Non Ferrous based  $O_2$  scavenging film Ham Zero order plot of  $a^*$  vs. time (7 days) with 95 % confidence limits calculation.

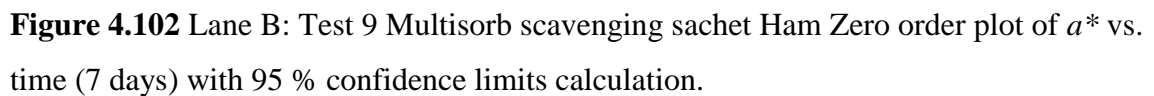
[illegible]

**Table 4.155** Test 9  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the Ferrous based O<sub>2</sub> scavenging sachet (Multisorb<sup>®</sup>) package in Lane A

1. Raw Data:															
# data pairs	Total=	5	This is automatically counted												
Y units	a*														
X units	days														
STATISTICS															
2. Calculation Note after entering Y and X you need to pull down formulas in each column from top to last entry															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
18.39	1.0	338.19	18.39	17.32	1.00	18.39	17.32	1.14	5.76	18.39	1.00	19.96	14.69	5.26	17.32
15.85	2.0	251.22	15.85	17.49	4.00	15.85	17.49	2.70	1.96	31.70	4.00	19.57	15.42	4.14	17.49
18.31	3.0	335.26	18.31	17.66	9.00	18.31	17.66	0.42	0.16	54.93	9.00	19.41	15.91	3.49	17.66
17.57	4.0	308.70	17.57	17.83	16.00	17.57	17.83	0.07	0.36	70.28	16.00	19.61	16.04	3.57	17.83
18.52	7.0	342.99	18.52	18.33	49.00	18.52	18.33	0.03	12.96	129.64	49.00	21.79	14.88	6.90	18.33
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average

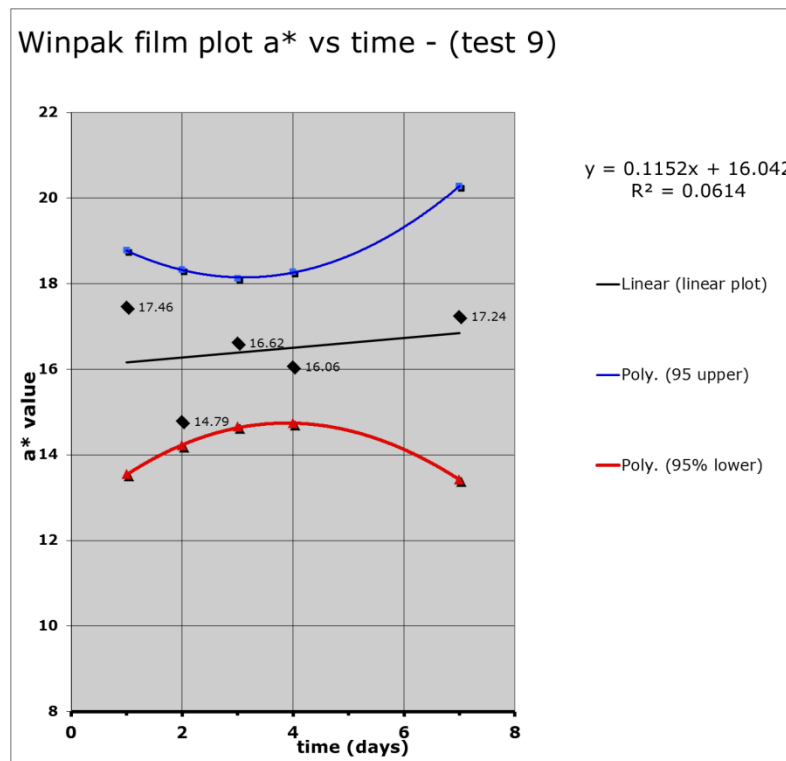


**Figure 4.101** Lane A: Test 9 Ferrous based O<sub>2</sub> scavenging sachet Ham Zero order plot of  $a^*$  vs. time (7 days) with 95 % confidence limits calculation

[illegible]

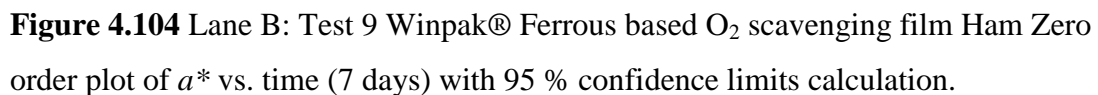
**Table 4.157** Test 9  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the Winpak® Ferrous based O<sub>2</sub> scavenging film package in Lane A

<b>1. Raw Data:</b>														
# data pairs	Total=	5	This is automatically counted											
Y units	a*													
X units	days													
<b>2. Calculations:</b>														
Note after entering Y and X you need to pull down formulas in each column from top to last entry (y1-yes)*2														
Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	yi estimate	(y1-yes)^2	(xi-xave)^2	xi*y1	X^2	y 95%UL	y 95%LL	Delta
17.46	1.0	304.85	17.46	16.16	1.00	17.46	16.16	1.70	5.76	17.46	1.00	18.77	13.54	5.23
14.79	2.0	218.74	14.79	16.27	4.00	14.79	16.27	2.20	1.96	29.58	4.00	18.33	14.21	4.12
16.62	3.0	276.22	16.62	16.39	9.00	16.62	16.39	0.05	0.16	49.86	9.00	18.12	14.65	3.47
16.06	4.0	257.92	16.06	16.50	16.00	16.06	16.50	0.20	0.36	64.24	16.00	18.28	14.73	3.55
17.24	7.0	297.22	17.24	16.85	49.00	17.24	16.85	0.15	12.96	120.68	49.00	20.28	13.42	6.86
Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	yi estimate	(y1-yes)^2	(xi-xave)^2	xi*y1	X^2	y 95%UL	y 95%LL	Delta
<div> <div>slope= 0.1152</div> <div>intercept= 16.0424</div> <div>rsq= 0.0614</div> <div>± 95% slope 0.8267</div> <div>k upper 0.9419</div> <div>k lower -0.7115</div> </div> <div> <div>Standard Error 1.20</div> <div>Sum (yi-yes) 1548.44</div> <div>n 5.00</div> <div>t 95%,2,n-2= 3.18</div> <div>x average = 3.40</div> <div>Sum (xi-xav) 90.56</div> <div>(Sum x)^2 289.00</div> <div>Sum(y^2) 1354.96</div> <div>sum y 82.17</div> <div>Sum (xi*y1) 281.82</div> <div>sum x 17.00</div> <div>sum (X^2) 79.00</div> </div>														
<div>Equations</div> <div>Y = 16.0424 + 0.1152 * time</div>														



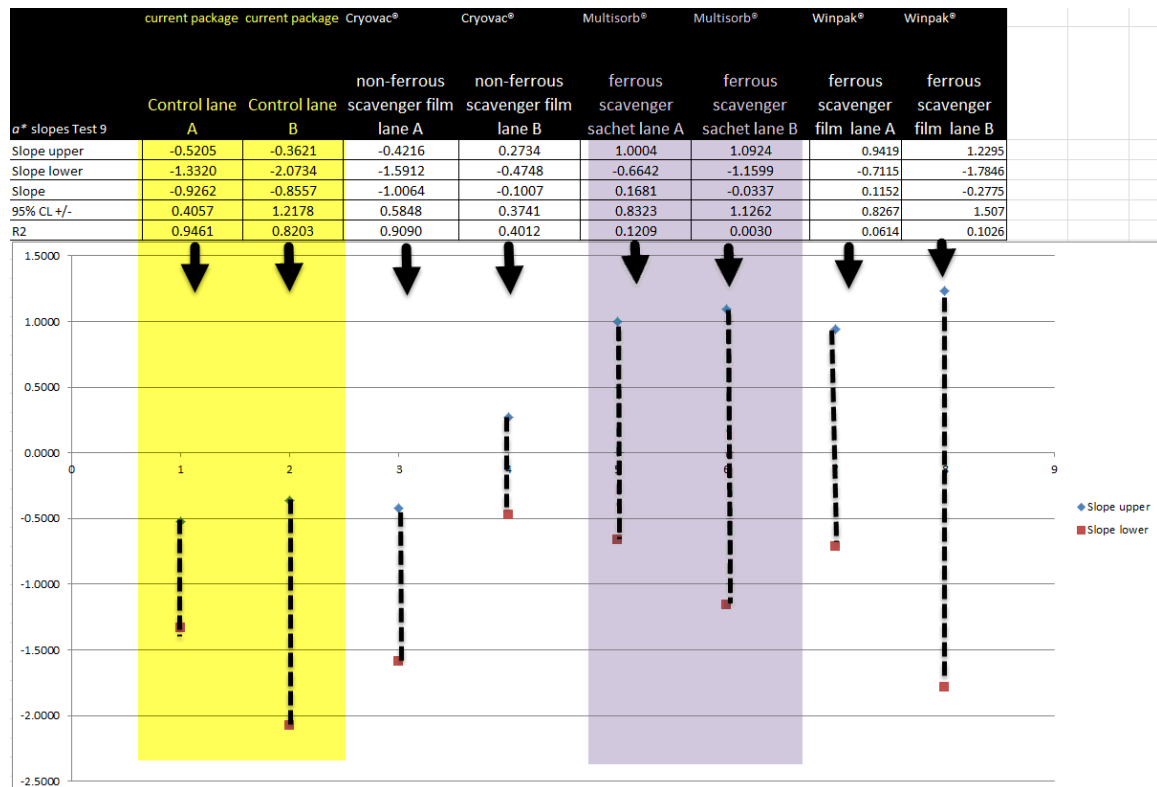
**Figure 4.103** Lane A: Test 9 Winpak® Ferrous based O<sub>2</sub> scavenging film Ham Zero order plot of  $a^*$  vs. time (7 days) with 95 % confidence limits calculation.

1. Raw Data:																
# data pairs	Total=	5	This is automatically counted													
Y units	a*	lane B Winpak														
X units	days															
STATISTICS																
2. Calculation: Note after entering Y and X you need to pull down formulas in each column from top to last entry rd(yi-yes)*2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
	20.22	1.0	408.85	20.22	18.07	1.00	20.22	18.07	4.60	5.76	20.22	1.00	22.84	13.31	9.53	18.07
	14.79	2.0	218.74	14.79	17.80	4.00	14.79	17.80	9.04	1.96	29.58	4.00	21.55	14.04	7.50	17.80
	17.47	3.0	305.20	17.47	17.52	9.00	17.47	17.52	0.00	0.16	52.41	9.00	20.68	14.36	6.32	17.52
	18.03	4.0	325.08	18.03	17.24	16.00	18.03	17.24	0.62	0.36	72.12	16.00	20.47	14.01	6.46	17.24
	16.53	7.0	273.35	16.53	16.41	49.00	16.53	16.41	0.02	12.96	115.73	49.00	22.66	10.16	12.50	16.41
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
	<div style="display: flex; justify-content: space-between;"> <div> <p>slope= -0.2775</p> <p>intercept= 18.3523</p> <p>rsq= 0.1026</p> <p>± 95% slope 1.5070</p> <p>k upper 1.2295</p> <p>k lower -1.7846</p> </div> <div> <p>Equations</p> <p>Y = 18.3523 - 0.2775 * time</p> </div> <div> <p>Standard Error 2.18</p> <p>Sum (yi-yes) 2035.13</p> <p>n 5.00</p> <p>t 95% 2,n-2 3.18</p> <p>x average = 3.40</p> <p>Sum (xi-xav) 90.56</p> <p>(Sum x)^2 289.00</p> <p>Sum(y^2) 1531.23</p> <p>sum y 87.04</p> <p>Sum (xi*yi) 290.06</p> <p>sum x 17.00</p> <p>sum (X^2) 79.00</p> </div> </div>															



Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments is provided in Table 4.159. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 4.159**  $a^*$  rate constant ( $k$ ) upper and lower for all applications in Test 9 lane A as established by Labuza' Reaction kinetics shelf life model



The ferrous scavenger sachet (Multisorb® D-50) did not produce as narrow of a prediction range of slopes as seen in test 7 where the slope  $\pm$  95% confidence level was 0.1733 (lane A) and 0.3434 (lane B) compared to 0.8323 (lane A) and 1.1262 (lane B) in this test (Table 4.159), but it did demonstrate a much smaller percentage of predicted overlap compared to the control (Table 4.159) with a minimum predicted value for the scavenger of -0.6642 in lane A compared to an upper predicted value for the control of -

0.5205, and a much greater likelihood of a positive slope (increasing redness) over time. The control package predicted only negative slopes (decreasing redness) over time. The lane A ferrous scavenger film ( $a^*$  slope =  $0.1681 \pm 0.8323$ ) was similar to the sachet ( $a^*$  slope =  $0.1152 \pm 0.8267$ ), but had a broader range of predicted outcomes in lane B ( $a^*$  slope =  $-0.2775 \pm 1.507$ ) demonstrating less consistency.

Part of the differences observed in predicted slopes from previous tests may be attributed to limiting the evaluations to one week. As previously observed,  $a^*$  values typically improve over time for most treatments as a result of moisture loss and potential condition changes that favor redevelopment of nitrosylhemochrome (pH, residual nitrate).

Evaluating the data over 7 days compared to 30 days suggests that performance observations may vary from week to week. In this study, the  $R^2$  for the controls and lane A non-ferrous based scavenger indicate a better fit of the data (Figures 4.97 – 4.100), demonstrating a consistent decline in slope from day 1 to day 7. Because the  $a^*$  values often show improvement by day 30, the  $R^2$  outcomes tend to indicate a poor fit of data. While it is convenient to have a better fit of data and a clear trend demonstrated in week 1, the solution needs to apply to the 30 day shelf life as it is unknown (and likely highly variable) when the sandwich is purchased and consumed within the 30 day refrigerated shelf life. Based on the guaranteed sale, the only known is that approximately 5% of the Ham & cheese sandwiches in Market are pulled after 30 days of refrigeration (E.A. Sween Co.)

Regardless of the observed difference in predicted  $a^*$  slope performance for all treatments over time above, there is overlap for all and are not statistically different from each other.

#### **4.9.5 – $L^*$ scores Test 9**

The variability of  $L^*$  values across all treatments over time is large (Table 4.160).



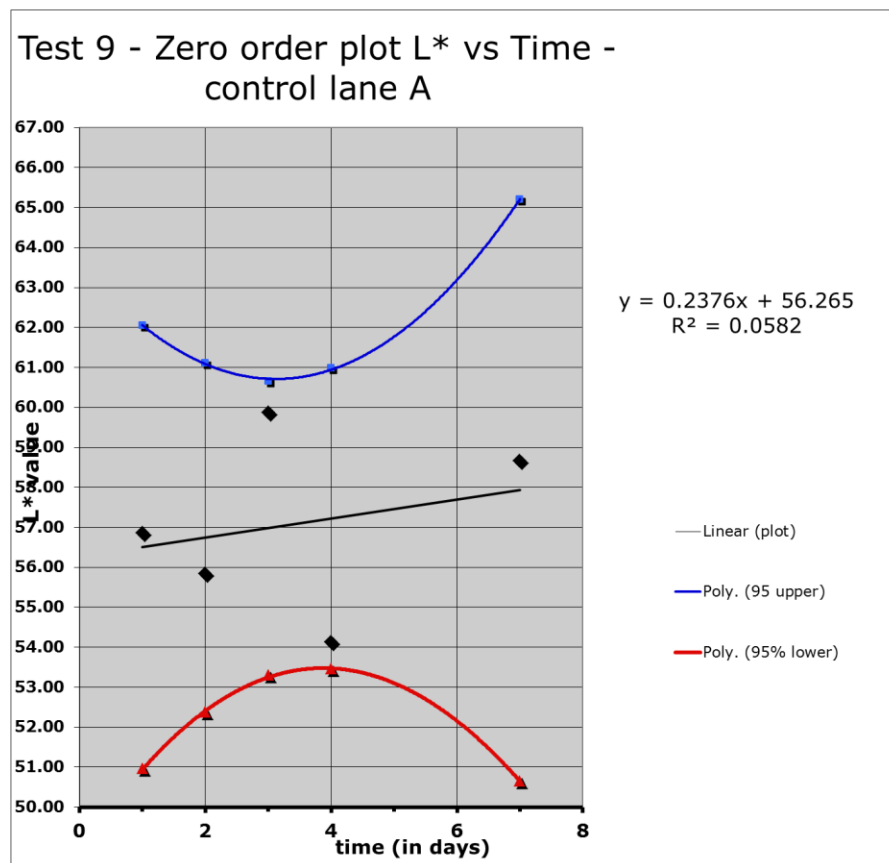
**Table 4.160**  $L^*$  color scores both test and control film Test 9. Values in red indicate packages that were identified as leakers (improper seal) with high oxygen in the headspace, but are not included in the minimum, maximum or statistical calculations

day	Control lane A $L^*$	Control lane B $L^*$	Winpak lane A $L^*$	Winpak lane B $L^*$	Cryovac lane A $L^*$	Cryovac lane B $L^*$	Multisorb lane A $L^*$	Multisorb lane B $L^*$
1	56.87	53.40	57.87	54.31	58.27	57.44	56.94	57.44
2	55.85	58.94	57.98	62.29	59.35	55.44	59.31	60.51
3	59.87	59.81	57.46	57.56	61.38	57.82	56.35	61.10
4	54.12	60.80	61.24	57.31	61.03	59.06	57.91	55.29
7	58.66	59.87	55.55	59.12	59.28	56.93	54.59	57.81
min	54.12	53.40	55.55	54.31	58.27	55.44	54.59	55.29
max	59.87	59.87	61.24	62.29	61.38	59.06	59.31	61.10
range	5.74	6.48	5.69	7.98	3.12	3.63	4.72	5.81

The largest  $\Delta L^*$  over time occurs in the Ferrous based scavenging film (Winpak®) ( $\Delta L^*=5.69, 7.98$ ), with the narrowest  $\Delta L^*$  in the non-ferrous based scavenger (Cryovac) ( $\Delta L^*=3.12, 3.63$ ). The performance of the Ferrous based scavenger sachet (Multisorb) and control treatments were most similar to the Ferrous based scavenging film (Winpak®). All maximum value observed for all treatments were similar. Entering the  $L^*$  values from Table 4.160 above into the kinetics data input sheet (Tables 4.161 – 4.168) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey or concentration of pigments (which is also an indication of moisture loss).

**Table 4.161** Test 9  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the control package in Lane A

1. Raw Data:														
# data pairs		Total=	5	This is automatically counted										
Y units	L*	Lane A	control											
X units	days													
STATISTICS														
2. Calculati														
Note after entering Y and X you need to pull down formulas in	each column from top to last entry rd(yi-yes)^2													
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte
56.87	1.0	3233.82	56.87	56.50	1.00	56.87	56.50	0.13	5.76	56.87	1.00	62.05	50.95	11.10
55.85	2.0	3118.85	55.85	56.74	4.00	55.85	56.74	0.80	1.96	111.69	4.00	61.11	52.37	8.74
59.87	3.0	3584.02	59.87	56.98	9.00	59.87	56.98	8.35	0.16	179.60	9.00	60.66	53.30	7.36
54.12	4.0	2929.34	54.12	57.22	16.00	54.12	57.22	9.56	0.36	216.49	16.00	60.98	53.45	7.53
58.66	7.0	3441.00	58.66	57.93	49.00	58.66	57.93	0.54	12.96	410.62	49.00	65.21	50.65	14.56
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta
												Standard Error	2.54	
												Sum (yi-yes)	15847.95	
												n	5.00	
												t 95%,2,n-2=	3.18	
												x average =	3.40	
												Sum (xi-xav)	79.00	
												(Sum x)^2	289.00	
												Sum(y^2)	16307.02	
												sum y	285.36	
												Sum (xi*yi)	975.27	
												sum x	17.00	
												sum (X^2)	79.00	
Equations														
Y = 56.2647    0.2376    * time														



**Figure 4.105** Lane A: Test 9 control package Ham Zero order plot of  $L^*$  vs. time (7 days) with 95 % confidence limits calculation

1. Raw Data:			
# data pairs Total=	4	This is automatically counted	
Y units	L*	Lane B	control
X units	days		

STATISTICS															
2. Calculati		Note after entering Y and X you need to pull down formulas in	each column from top to last entry rdyi-yesy)*2												
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yesy)*2	(xi-xave)^2	xi*yi	X^2	y 95%JL	y 95%4.L	Delta	predicte average
53.40	1.0	2851.20	53.40	56.30	1.00	53.40	56.30	8.42	5.06	53.40	1.00	65.09	47.51	17.58	56.30
58.94	2.0	3474.32	58.94	57.06	4.00	58.94	57.06	3.56	1.56	117.89	4.00	64.19	49.93	14.26	57.06
59.81	3.0	3577.24	59.81	57.82	9.00	59.81	57.82	3.98	0.06	179.43	9.00	64.11	51.53	12.58	57.82
59.87	7.0	3584.82	59.87	60.85	49.00	59.87	60.85	0.96	14.06	419.11	49.00	72.90	48.81	24.09	60.85
		0.00	0.00	55.54	0.00	0.00	55.54	3084.56	10.56	0.00	0.00	66.43	44.65	21.79	55.54
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yesy)*2	(xi-xave)^2	Xi*Yi	X^2	y 95%JL	y 95%4.L	Delta	predicte average

slope=	0.7591
intercept=	55.5388
rsq=	0.4142
± 95% slope	2.7448
k upper	3.5039
k lower	-1.9857

Equations	
Y = 55.5388	0.7591 * time

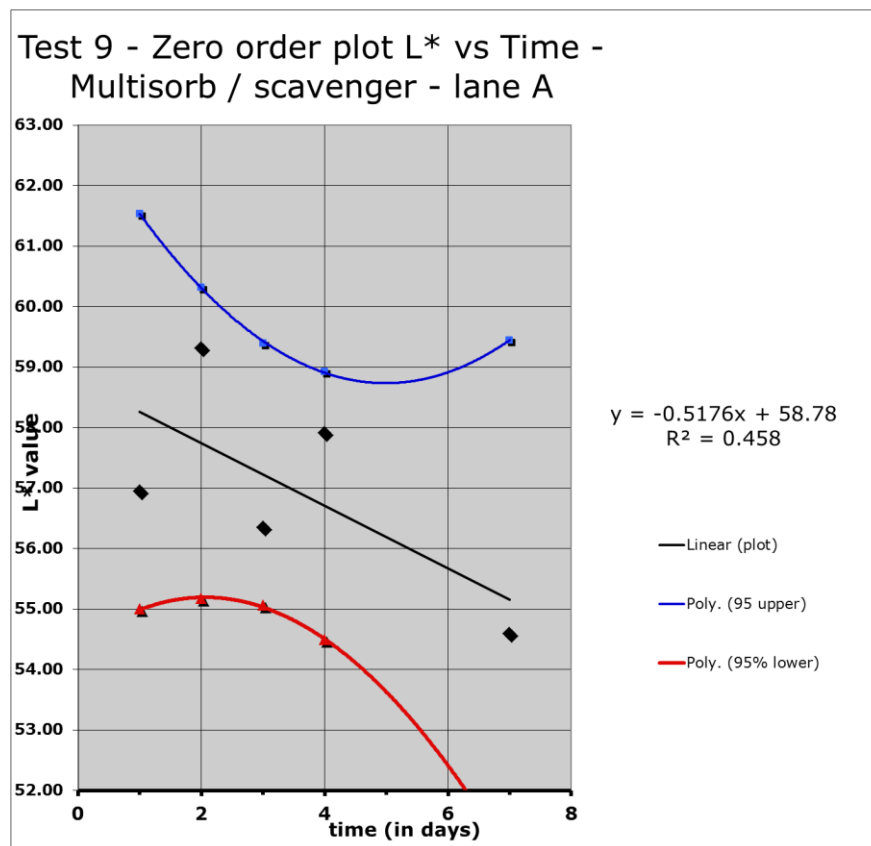
  

Standard Er	2.91
Sum (yi-yes	18524.28
n	4.00
t 95% 2,n-2=	4.30
x average =	3.25
Sum (xi-xav	84.13
(Sum x)^2	169.00
Sum(y^2)	13487.57
sum y	232.02
Sum (xi*yi)	769.83
sum x	13.00
sum (X^2)	63.00



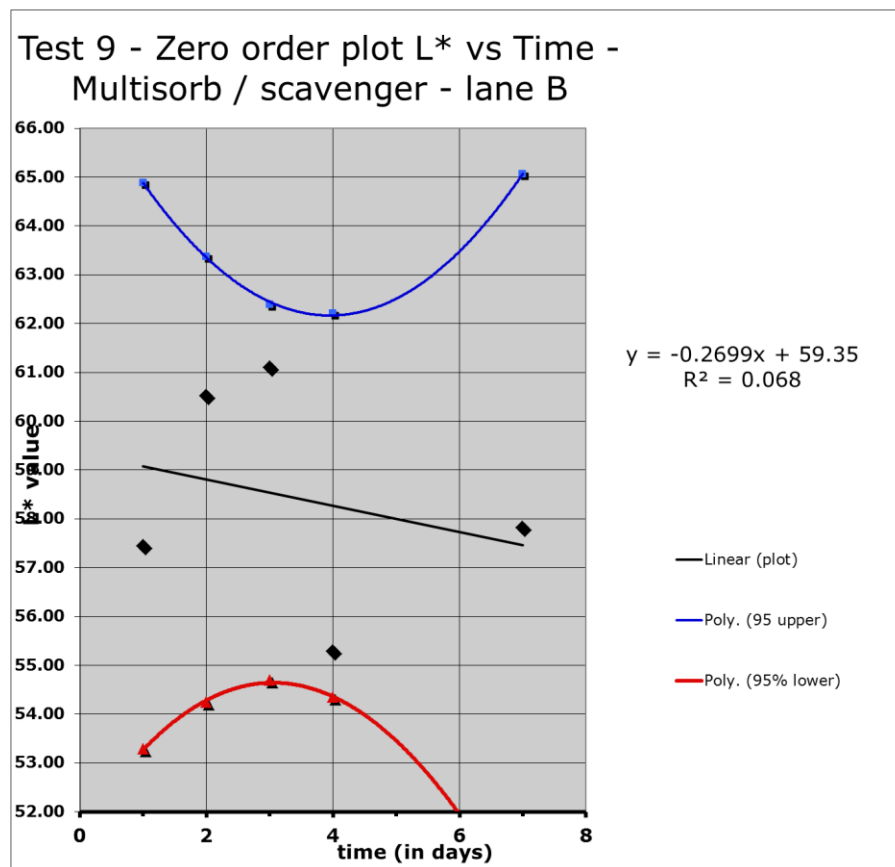
**Table 4.163** Test 9  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the Multisorb® scavenger package in Lane A

1. Raw Data:															
# data pairs	Total=	5	This is automatically counted												
Y units	L*	Multisorb lane A													
X units	days														
STATISTICS															
2. Calculati															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte
56.94	1.0	3242.54	56.94	58.26	1.00	56.94	58.26	1.74	5.76	56.94	1.00	61.53	54.99	6.54	58.26
59.31	2.0	3517.28	59.31	57.74	4.00	59.31	57.74	2.44	1.96	118.61	4.00	60.32	55.17	5.15	57.74
56.35	3.0	3175.32	56.35	57.23	9.00	56.35	57.23	0.77	0.16	169.05	9.00	59.40	55.06	4.34	57.23
57.91	4.0	3353.95	57.91	56.71	16.00	57.91	56.71	1.45	0.36	231.65	16.00	58.93	54.49	4.43	56.71
54.59	7.0	2979.70	54.59	55.16	49.00	54.59	55.16	0.32	12.96	382.11	49.00	59.44	50.87	8.58	55.16
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte
slope=												Standard Error	1.50		
intercept=												Sum (yi-yes)	20737.16		
rsq=												n	5.00		
± 95% slope												t 95%, 2, n-2=	3.18		
k upper												x average =	3.40		
k lower															
Equations												Sum (xi-xav)	90.56		
Y = 58.7799 - 0.5176 * time												(Sum x)^2	289.00		
												Sum(y^2)	16268.80		
												sum y	285.10		
												Sum (xi*yi)	958.37		
												sum x	17.00		
												sum (X^2)	79.00		



**Figure 4.107** Lane A: Test 9 Multisorb® scavenger package Ham Zero order plot of  $L^*$  vs. time (7 days) with 95 % confidence limits calculation.

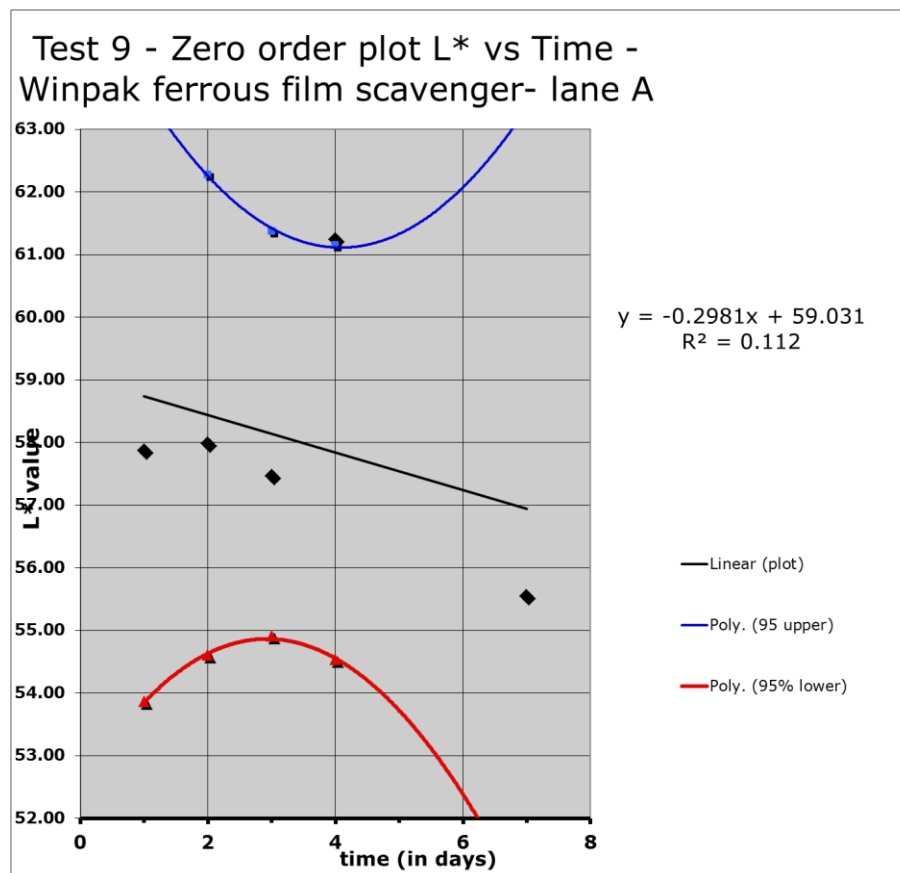
<b>1. Raw Data:</b>															
# data pairs Total=	5 This is automatically counted														
Y units	L*	Multisorb lane B													
X units	days														
<b>STATISTICS</b>															
<b>2. Calculate Note after entering Y and X you need to pull down formulas in each column from top to last entry r(dy-yes)*^2</b>															
(xi-xave)^2	Xi*yi	X^2	y y95%UL	y y95%LL	Delta	predicte average									
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yest)^2	(xi-xave)^2	Xi*Yi	X^2	y y95%UL	y y95%LL	Delta	predicte average
57.44	1.0	3299.74	57.44	59.08	1.00	57.44	59.08	2.68	5.76	57.44	1.00	64.88	53.28	11.60	59.08
60.51	2.0	3661.86	60.51	58.81	4.00	60.51	58.81	2.90	1.96	121.03	4.00	63.38	54.24	9.13	58.81
61.10	3.0	3733.62	61.10	58.54	9.00	61.10	58.54	6.57	0.16	183.31	9.00	62.39	54.69	7.69	58.54
55.29	4.0	3056.98	55.29	58.27	16.00	55.29	58.27	8.88	0.36	221.16	16.00	62.20	54.34	7.87	58.27
57.81	7.0	3342.38	57.81	57.46	49.00	57.81	57.46	0.12	12.96	404.69	49.00	65.07	49.85	15.21	57.46
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yest)^2	(xi-xave)^2	Xi*Yi	X^2	y y95%UL	y y95%LL	Delta	predicte average
slope=	-0.2699											Standard Error	2.66		
intercept=	59.3503											Sum (yi-yes)	21155.94		
r sq=	0.0680											n	5.00		
+ 95% slope	1.8341											t 95%,2,n-2	3.18		
k upper	1.5642											x average =	3.40		
k lower	-2.1040														
												Sum (xi-xav	90.56		
												(Sum x)^2	289.00		
												Sum(y^2)	17094.58		
												sum y	292.16		
												Sum (xi*yi)	987.63		
												sum x	17.00		
												sum (X ^2)	79.00		
												Equations			
												Y = 59.3503 -0.2699 * time			



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**Table 4.165** Test 9  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the Winpak<sup>®</sup> ferrous scavenger film in Lane A

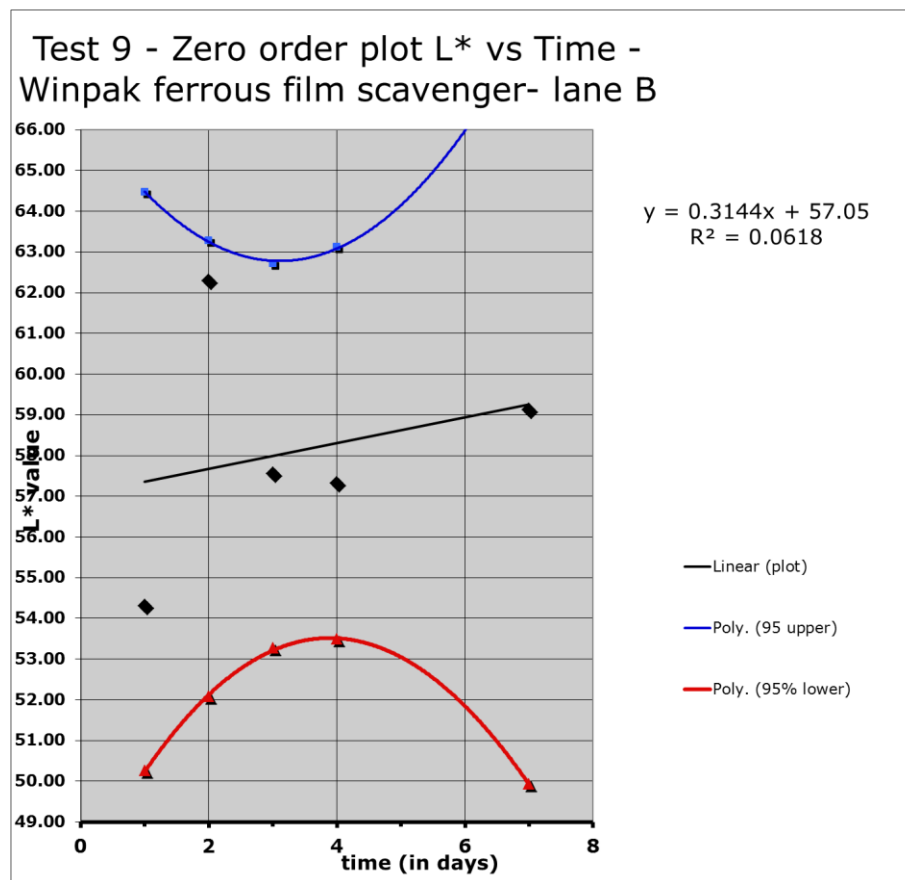
1. Raw Data:														
# data pairs	Total=	5	This is automatically counted											
Y units	L*	Winpak lane A												
X units	days													
STATISTICS														
2. Calculati														
Note after entering Y and X you need to pull down formulas in each column from top to last entry row (y1-yes)														
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
57.87	1.0	3348.55	57.87	58.73	1.00	57.87	58.73	0.75	5.76	57.87	1.00	63.61	53.86	9.75
57.98	2.0	3361.29	57.98	58.44	4.00	57.98	58.44	0.21	1.96	115.95	4.00	62.27	54.60	7.68
57.46	3.0	3301.65	57.46	58.14	9.00	57.46	58.14	0.46	0.16	172.38	9.00	61.37	54.90	6.47
61.24	4.0	3750.34	61.24	57.84	16.00	61.24	57.84	11.57	0.36	244.96	16.00	61.14	54.53	6.61
55.55	7.0	3085.43	55.55	56.94	49.00	55.55	56.94	1.96	12.96	388.83	49.00	63.34	50.55	12.78
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta
predicted average														



**Figure 4.109** Lane A: Test 9 Winpak<sup>®</sup> ferrous scavenger film Ham Zero order plot of  $L^*$  vs. time (7 days) with 95 % confidence limits calculation

**Table 4.166** Test 9  $L^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the Winpak<sup>®</sup> ferrous scavenger film in Lane B

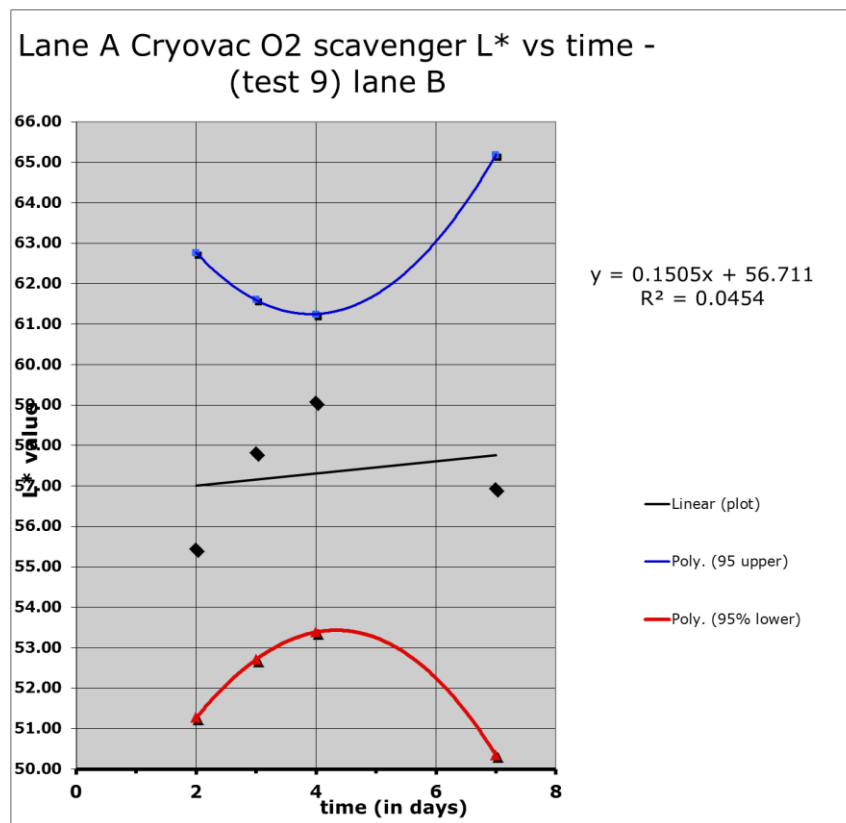
1. Raw Data:																	
# data pairs Total=	5 This is automatically counted																
Y units	L* Winpak lane B																
X units	days																
STATISTICS																	
2. Calculati																	
Note after entering Y and X you need to pull down formulas in	each column from top to last entry rdy(yi-yes)*2																
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte		
54.31	1.0	2949.21	54.31	57.36	1.00	54.31	57.36	9.35	5.76	54.31	1.00	64.48	50.25	14.22	57.36		
62.29	2.0	3880.04	62.29	57.68	4.00	62.29	57.68	21.27	1.96	124.58	4.00	63.28	52.08	11.20	57.68		
57.56	3.0	3313.15	57.56	57.99	9.00	57.56	57.99	0.19	0.16	172.68	9.00	62.71	53.28	9.43	57.99		
57.31	4.0	3284.82	57.31	58.31	16.00	57.31	58.31	0.99	0.36	229.25	16.00	63.13	53.48	9.65	58.31		
59.12	7.0	3495.57	59.12	59.25	49.00	59.12	59.25	0.02	12.96	413.86	49.00	68.58	49.92	18.65	59.25		
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte		
slope=												0.3144					
intercept=												57.0496					
rsq=												0.0618					
± 95% slope												2.2488					
k upper												2.5632					
k lower												-1.9344					
Equations																	
Y = 57.0496												0.3144		* time			



**Figure 4.110** Lane B: Test 9 Winpak<sup>®</sup> ferrous scavenger film Ham Zero order plot of  $L^*$  vs. time (7 days) with 95 % confidence limits calculation



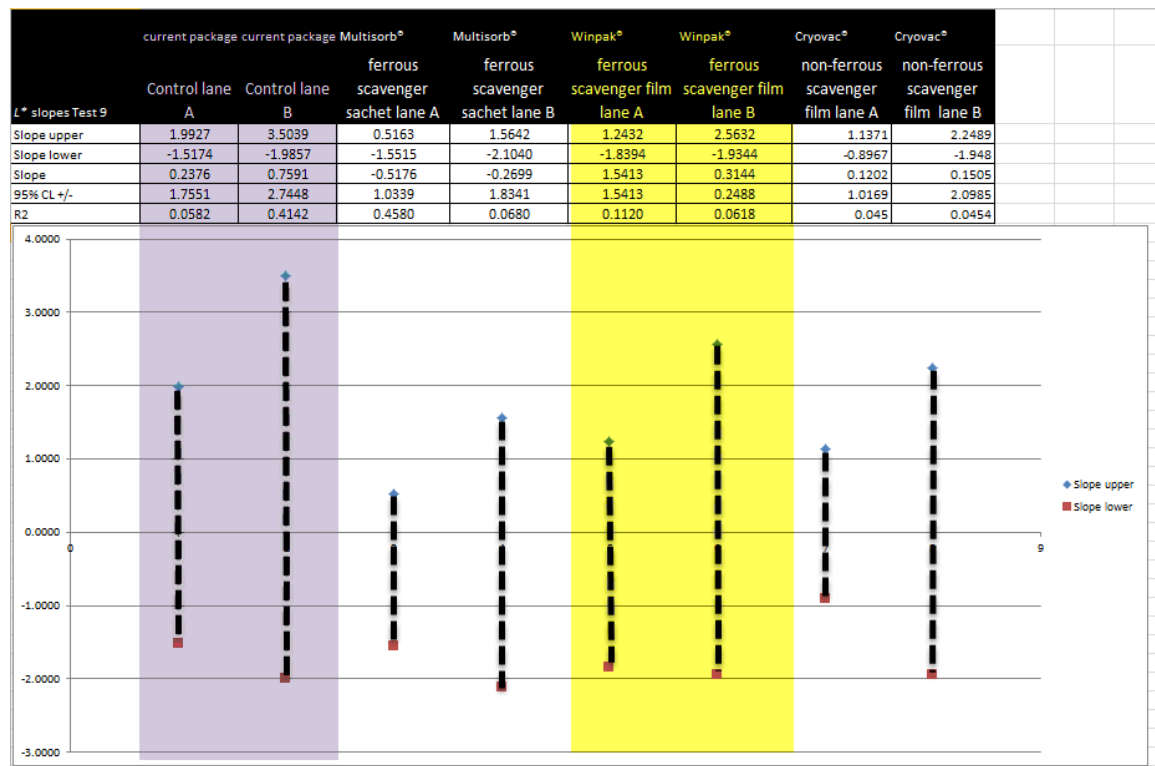


[illegible]

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Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges ( $+k$  for lightening over the shelf life,  $-k$  for darkening at  $\pm$  95% CL) between treatments is provided in Table 4.169.

**Table 4.169**  $L^*$  parameter rate constant ( $k$ ) upper and lower for all applications in Test 9 all lanes as established by Labuza' Reaction kinetics shelf life model.



All treatments demonstrated a large range in variability of predicted slope performance at the 95% confidence level. The performance of all three test package is similar, with a smaller range of predicted outcomes to the control. However, there is overlap of predicted outcomes for all treatments which makes the performance for each treatment not statistically different.

#### **4.9.6 - Visual appearance of the ham Test 9**

By day 2 of the study, the Cryovac sample and control sample had signs of visual discoloration. (Appendix I.1) The Ferrous based film sample had slight discoloration at day 3 (Appendix I.3) and the visual of the ham with the sachet in the package had no brown color development on days 1-7. (Appendix I.1 – I.5)

#### **4.9.7 – Cooler temperatures Test 9**

The coolers utilized were the same as Test 8. The settings were not changed. Refrigerated temperatures were not tracked in this study.

#### **4.9.8 – Conclusions Test 9**

Both the ferrous based oxygen scavenging sachet and film visually resulted in pinker ham with absence of significant discoloration. While  $a^*$  value predicted slopes over time do not support a statistical difference over time, the predicted  $a^*$  values for both ferrous based applications were consistently higher than the control and non-ferrous application, and the ferrous sachet demonstrated very little overlap in lane A of predicted outcomes to the control. While the sachet has yet to achieve true zero percent oxygen in the headspace, and the  $a^*$  value data does not support statistical differences, the lack of development of visual discoloration on the ham has been impressive and worth further investigation with consumer input. The lack of visual discoloration has been repeatable for the D-50 oxygen scavenger (Test 7 and 9). In addition to validating, if consumers visually see a difference in the color of the cured ham over time, gauging consumer attitude toward the sachet in contact with the sandwich will be important to long term viability. The sachet is not visible in the sealed package. If the sachet, once the sandwich is opened, negatively influences repeat purchases, this decreases the viability of it as a solution.

While the results of the ferrous based scavenging film were not as strong as the sachet on  $a^*$  and  $L^*$  values, this may be viewed as a more consumer friendly option and worth investigating consumer opinion.

There are four potential causes (or combinations of) for the ferrous based scavengers not achieving 0% O<sub>2</sub> in a MAP environment including 1) Not enough air flow around the sachet, 2) Greater than 30 cc needed to be removed (which is possible if more significant trapped air was present or if the Multivac process did not remove sufficient quantities of O<sub>2</sub>, but neither are likely based on previous testing), 3) Not enough time prior to freezing for the sachet to remove O<sub>2</sub> (as the O<sub>2</sub> scavenging reaction becomes extremely slow at freezing temperatures) and 4) The presence of CO<sub>2</sub> inhibiting oxygen absorption.

This test was not designed to establish which of the four potential causes resulted in O<sub>2</sub> scavenger packages with residual oxygen. Future tests could be set up to establish if better air flow around the sachet or removal of the carbon dioxide creates better O<sub>2</sub> % results. However, removal of CO<sub>2</sub> creates potential food safety concerns as the bacteriostatic effect of CO<sub>2</sub> provides some measures to prevent growth of facultative or anaerobic psychotropic pathogens (Sofos, 1993). Additionally, the best option to improving air flow would be to attach the scavenger to the top film creating the potential of it being more visible to the consumer, but creates a challenge of how to attach it to the package without adding significant cost. The freezing process creates an issue for ferrous based oxygen scavenging as a minimum 40% relative humidity in the package is required for scavenging to occur. Another potential test could be to store the sandwiches in dark refrigeration for 24 hours prior to freezing, but available refrigerated space is required in a manufacturing facility to accomplish this.

## **5 Consumer and follow up study Tests 10-11**

### **5.1 Ferrous based oxygen scavenger packaging solutions and consumer study (Test 10)**

#### **5.1.1 Overview of Test 10**

In the previous Tests 7 and 9, the Multisorb<sup>®</sup> D-50 O<sub>2</sub> scavenger sachet demonstrated that it was capable of further lowering oxygen (to near zero) in a Modified Atmosphere Package (MAP) compared to MAP process only, even when the product is placed in frozen storage following assembly and packaging. While the O<sub>2</sub> scavenger sachet  $a^*$  and  $L^*$  color scores in previous tests were not statistically different compared to MAP only, the  $a^*$  slope of the line as predicted by the chemical kinetics zero order reaction spreadsheet at the 95% confidence level has demonstrated a greater likelihood of positive slopes over time (increased redness), and has often supported a narrower range of potential slopes compared to MAP only (creating a better predictability in outcome). Additionally, the appearance of the ham with the D-50 cc scavenger has demonstrated no obvious visual discoloration where the MAP only ham in the same time interval has discoloration characteristic of photo-oxidation. Based on this evidence, the D-50 solution will be presented to consumers in this final study to establish preference of the sandwich appearance with and without the scavenger. If the consumer consistently prefers the color of the ham in the scavenger protected MAP packages, it would validate visual observations and thus use of O<sub>2</sub> scavenger sachets as a practical solution for the sandwich industry to slow meat discoloration is recommended, and would justify the added cost of the solution to the prepackaged sandwich. The economics of adding a spooled (to automate placement) D-50 O<sub>2</sub> scavenger sachet to a MAP package depends on volume purchased and can range from approximately \$0.027- \$0.032 per sachet. The sachet solution needs to improve sales by eliminating creation of an unacceptable product at point of sale, or meaningfully impact repeat purchase intent, otherwise profitability decreases or retail price increases.

The Winpak<sup>®</sup> ferrous based oxygen scavenging film in test 9 also demonstrated visual color improvements to MAP only and a greater potential for a positive  $a^*$  slope over time (although a greater potential for negative slope compared to the sachet). Given that this could be viewed as a more consumer friendly option to a sachet, the product was included in the consumer test to better understand consumer opinion towards the appearance of the package.

Using Food Perspectives Inc. to facilitate, 110 consumers were recruited to conduct a consumer preference test between the Deli Express<sup>®</sup> Ham and Cheese wedge shape sandwich (control package) with and without the Multisorb<sup>®</sup> D-50 cc O<sub>2</sub> scavenger sachet. Consumers were also shown sandwich packaged with Winpak<sup>®</sup> oxygen scavenging film (grey tint) and asked for opinions, but not preference. The objectives for the consumer study were 1) Determine which ham and cheese sandwich is preferred between the current (control with  $\leq 0.5\%$  targeted residual oxygen) and prototype (oxygen scavenger sachet included) packaging pairs and the degree of preference (at multiple ages during the shelf life (day 4, 7, and 30)). 2) Determine if the product appearance in the new packaging solution will meaningfully increase purchase interest and liking of the ham and cheese wedge sandwich. 3) To understand consumer's opinions toward oxygen scavengers (both film and sachet).

Historically, the United States (US) market has been slow to use oxygen scavengers with Japan leading the market followed by Western Europe (Pira International Ltd., 2009). The majority of the sales in the US are in pharmaceutical and food (roughly half and half) with less than 5% to other areas (like electronics). Globally, the majority of oxygen scavenger sales in food are for fresh and processed meats and beer. For beer, the scavenger is incorporated into the bottle, while fresh and processed meats use the sachet. Very few studies with oxygen scavengers are available that pair quantitative data (like  $L^*a^*b^*$  analysis) with consumer input regarding use of an oxygen scavenger. Several studies used trained color panels to score the color of the meat. No research was found for sandwiches regarding use of oxygen scavengers or related to the development of meat discoloration and acceptability. Establishing acceptance of the scavenger to the consumer is important to defining it as a viable business solution. If addition of the

sachet decreases repeat purchase intent of the sandwich, an understanding of how much (compared to decreased purchase intent because of meat discoloration) would be required.

To correlate quantitative data ( $L^*a^*b^*$ ) to qualitative data (consumer input), flat ham format sandwiches were created from the same production lots of ham, cheese and bread as were used to create the retail wedge packages for the consumer test. Using the same protocol from previous tests,  $L^*$  and  $a^*$  color analysis, package headspace oxygen levels, storage temperature, and visual observations were gathered over the course of a 30 day refrigerated shelf life.

## **5.1.2 Methods and Materials Test 10**

### **5.1.2a Methods and Materials in Test 10, sample preparation and storage**

Six Beverage Air coolers (Model # LV27 c) with fluorescent lighting were used in this study. New fluorescent bulbs (Buyers Choice cool white 32 watt fluorescent – section 3.18) were installed in each cooler to eliminate any age inconsistency between bulbs. This test consisted of both the retail wedge sandwich format for consumer evaluation, and the flat sandwich format for  $L^*a^*b^*$  color analysis and visual interpretation. Both packaging formats were produced at the same time using separate R530 Multivac equipment (designated Red Phoenix and Green Wolf at E.A. Sween Company). The control packaging used for the flat format package is as described in section 3.3 (Belmark clear non-forming film) and 3.4a (Curwood clear forming film). The consumer wedge packaging used is as described in section 3.3 (Belmark clear non-forming film) and 3.4b (Curwood black forming film). For the test packaging, two treatments were prepared. The first was the control films with the Multisorb<sup>®</sup> D-50 oxygen scavenging sachet placed in both the consumer retail wedge samples and flat faced ham square package. The second was using Winpak<sup>®</sup> ferrous based oxygen scavenging film top (non-forming) film and bottom forming film as described in Test 9 in the wedge format.

All sandwiches were assembled and treated by MAP (Modified Atmosphere Packaging) with an 80% N<sub>2</sub> / 20% CO<sub>2</sub> blend (Materials and Methods section 3.15). The sandwich materials (ham, cheese and bread – section 3.1) were consistent (pulled from the same lot codes) across all samples / packaging formats (wedge or flat sandwiches). The ham and cheese was stored at approximately 0° C prior to slicing, and sliced on a Hobart slicer model 3913 (Troy, OH) prior to sandwich assembly. The bread was stored at room temperature (approximately 21° C) prior to assembly. The length of time from ham slicing to sandwich packaging was approximately ½ hour (Assembly area temperature is approximately 7°C). The age of the ham at the time of slicing was 28 days old. Immediately following package sealing, the sandwiches were placed in a corrugated box, taped shut and stored frozen (approximately 0°F). The approximate length of dark frozen storage for all sandwiches was 60 - 90 days (7/28/14 – 10/23/14)

For the cooler set, each cooler contained a mix of both packaging formats, with vertical lanes A and B loaded one sandwich deep on the front lip, and lane C loaded for the flat meat format (Figure 5.1).

Meat Discoloration Study 10											
			C	B	A	Cooler D			C7C	C7B	C7A
Cooler A			235	783	783				S7C	S7B	S7A
			235	426	426				235	397	397
lane A			235	783	783				235	840	840
lane B			235	426	426				235	397	397
lane C				783	783				235	840	840
empty				426	426				235	397	397
				783	783				235	840	840
				426	426						
Cooler B				783	783	Cooler E			972	397	397
				426	426				972	840	840
			C30C	C30B	C30A				972	397	397
			S30C	S30B	S30A				972	840	840
			C14C	C14B	C14A				C4C	C4B	C4A
			C14C	S14B	S14A				S4C	S4B	S4A
				559	559				C1C	C1B	C1A
				168	168				S1C	S1B	S1A
Cooler C			972	559	559	Cooler F			614	972	972
			972	168	168				614	235	235
			972	559	559				614	972	972
			972	168	168				614	235	235
			972	559	559				614	972	972
			972	168	168				614	235	235
			972	559	559				614	972	972
			972	168	168				614	235	235

**Figure 5.1** Cooler set up configuration for test 10. Eight shelves were utilized for a maximum of 24 sandwiches per cooler. The color coding above is by lane. Light blue represents lane A, light green represents lane B, and white represents lane C. Dark grey represents empty spaces in the cooler



To create multiple age pairs for same day evaluation, the sandwich thawing was staged to result in a 4, 7, and 30 day refrigerated pair for the wedges, and day 1, 4, 7, 14, and 30 for the flat meat package format on the day of the consumer study (Figure 5.2).

**Calendar**

7/28/2014	Produce samples in RTE	
9/27/2014	30	pull day 30 samples - 783, 426, all C30 & S30 flats
10/13/2014	14	pull day 14 samples - all C14 & S14 flats only
10/20/2014	7	pull all day 7 samples - C7 & S7 samples - 168,559, 972, 235,614
10/23/2014	4	pull day 4 samples - 397, 840, all C4 & S4 flats
10/26/2014	1	pull day 1 samples - C1 & S1 flats
10/27/2014	0	Consumer test day

**Figure 5.2** Calendar for production, sample staging, and consumer study

The consumers were presented with retail labeled products in the wedge format as it would appear on store shelves. The flat meat packages were not labeled to maximize the light exposure (Figure 5.3).



**Figure 5.3** Visual of package formats and product storage method Test 10 (The top two and bottom two rows represent sample pairs as viewed by the consumer) The middle 4 shelves represent the flat meat samples used for the  $L^*a^*b^*$  measurements

The consumer was asked to evaluate several age pairs of sandwiches where a control sample was paired with a scavenger sachet sample of the same age at days 4, 7, and 30 of a refrigerated shelf life. A list of age pairs is provided in Table 5.1.

**Table 5.1** Pair groups of ham and cheese sandwiches as presented to the consumer

Station	Description	Age of the sandwich	consumer code
1	Current (0.5% O2 target)	4	397
	Prototype (with sachet)	4	840
2	Current (0.5% O2 target)	7	168
	Prototype (with sachet)	7	559
3	Current (0.5% O2 target)	30	783
	Prototype (with sachet)	30	426

Five sets were prepared for each age pair allowing for multiple viewing stations. The ferrous based oxygen scavenging film was only utilized in peel off consumer sessions (Peel off session is a smaller discussion group (4 groups of n=5) providing qualitative

feedback that allows for a deeper exploration of responses and for general trends to be further identified). The consumer test method is described further in the next section.

### **5.1.2b Methods and Materials Consumer preference test design**

A consumer study was designed with a business objective of protecting the Deli Express<sup>®</sup> share of cured meat sandwiches purchased by addressing customers concerns about meat discoloration with active packaging improvements. The research objectives were to understand the overall liking of the sandwich appearance and color of the meat, and establish preference and purchase interest for sandwiches with and without an oxygen scavenging sachet included in the package (See Appendix J.16 for complete test design summary). The study design also allowed for a follow up discussion to better understand consumer's opinions toward oxygen scavengers (both film and sachet) and meat discoloration.

Only ferrous based scavenging solutions were considered based on visual improvements observed and better color stability results (a narrower range of  $a^*$  and  $L^*$  predicted slopes over time) from the previous Tests 1-9.

The consumer testing was conducted by Food Perspectives Inc. (FPI<sup>®</sup>). Founded in 1990 by Merry Jo Parker, FPI<sup>®</sup> focuses on designing and executing consumer tests on consumer-packaged goods, including food, beverages, household products, and industrial equipment. FPI<sup>®</sup> is located in Plymouth, MN and has more than 12,000 square feet available to conduct qualitative and quantitative consumer product research (Food Perspectives Inc., 2014).

The consumer test consisted of two components 1) Visual Central Location Test (CLT) and 2) A Qualitative Peel off study with blind product evaluation and assessment. In the CLT portion, a statistically relevant population of people (n=110) participated in a series of visual evaluation exercises. All respondents were prescreened from the FPI<sup>®</sup> database for age, gender, product usage within the category (sandwiches), and past participation (participation is limited to every 6 months to keep participant unbiased). Only those

within the project specified parameters were provided individual access to complete the online survey which screened further to identify actual prepackaged sandwich consumers, color blindness and other key requirements (Full screening details can be found in the Appendix J.12). A summary of the demographics for the study are listed in Figure 5.4 below.

## Demographics

Category	Classification	Percent
Gender	Male	52%
	Female	48%
Age	17 or younger	0%
	18–24	17%
	25–35	24%
	36–46	25%
	47–55	34%
	56–64	0%
	65 or older	
Ethnic Background	American Indian/Alaska Native	0%
	Asian	6%
	Black African American	9%
	Hispanic or Latino	1%
	Native Hawaiian/Other Pacific Islander	1%
	White/Caucasian	81%
	Other	1%

## Demographics – Continued

Category	Classification	Percent
Level of Schooling	Some grade school or high school	4%
	High school graduate	11%
	Some college or technical school	17%
	2-year college/technical degree	23%
	4-year college degree	29%
	Some graduate work	6%
	Graduate degree	10%
Household Size	1	15%
	2-3	46%
	4-5	33%
	6-7	5%
	8 or more	1%
Household Income	Under \$25,000 per year	5%
	\$25,000–\$39,999 per year	13%
	\$40,000–\$59,999 per year	19%
	\$60,000–\$79,999 per year	14%
	\$80,000–\$99,999 per year	18%
	\$100,000 or more per year	19%
	Prefer not to answer	12%

**Figure 5.4** Consumer preference testing demographics

All respondents in the CLT were presented with 3 pair groups of a control and scavenger packaged sandwich in the retail wedge format (aged 4, 7, and 30 days) and asked to complete a questionnaire for each sandwich in the pair (Appendix J.14). Each testing station consisted of one control and test sandwich, each sample was separated by a divider (Figure 5.5). Five identical stations were set up for each age pair group for a total of 15 stations. The pairs were rotated (balanced design) and presented in monadic sequential order (i.e. presented and evaluated one at a time).



**Figure 5.5** Visual of a consumer testing station in Test 10

Following the CLT, a peel off session was conducted. A peel off session is a smaller discussion group (4 groups of  $n=5$ ) providing qualitative feedback that allows for a deeper exploration of responses in the CLT and for general trends to be further identified. Peel off sessions are an effective way to gain a richer interpretation of data, while reducing cost in a larger study, and is viewed as supplemental to the CLT portion. In the peel off session, consumers were identified on arrival by conducting an articulation screen as well as availability to discuss their opinions on products further after the quantitative session. The peel-off groups were also balanced to represent individuals who selected the control (no scavenger) sample and the scavenger sample during the CLT test. Each Peel-off group was asked to open the control and test (scavenger) packages, remove the sandwich, taste, and discuss. They were also asked to visually access the oxygen scavenger film sample (which is grey tinted Figure 5.6) and

provide opinions on appearance. The peel off discussion was led by an independent moderator. (Moderator script in Appendix J.13)



**Figure 5.6** Grey tinted scavenger film (middle) alongside control (left) and scavenger sachet sample (right). The darker appearance of the middle package is a result of the ferrous powder in the sealant layer of the film.

As previously discussed, the current Deli Express ham and cheese wedge does not consistently result in discoloration in the current MAP package when exposed to light. Because meat discoloration development varies by package and consumers were presented with five different sandwiches (not pre-screened) as it would appear on the retail shelf nearest the light source, it was not guaranteed that the consumer would see a sample with significant discoloration in the CLT portion (but would see actual, representative retail examples). For this reason, a sample of a significantly discolored sandwich was presented for reaction during the peel off session. (Figure 5.7)





**Figure 5.7** Day 30 control sample in Test 10 as viewed by a consumer panel in the peel off session – oxygen % in the headspace was 0%. Sample was consistently rejected as spoiled by the peel off groups (Right side is as viewed by the consumer (packaged), left side is the appearance after removing the top film)

The study used liking questions (Figure 5.8) and meets expectations, purchase intent and preference questions (Figure 5.9); to gauge consumer opinion in the CLT.

xx. **OVERALL**, how well do you **LIKE** or **DISLIKE** the **APPEARANCE** of this product?

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike	Dislike	Dislike	Dislike	Neither Like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	Nor Dislike	Slightly	Moderately	Very Much	Extremely

xx. How much do you **LIKE** or **DISLIKE** the **MEAT COLOR** of this product?

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike	Dislike	Dislike	Dislike	Neither Like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	Nor Dislike	Slightly	Moderately	Very Much	Extremely

**Figure 5.8** Liking questions used in the CLT portion of the consumer test

xx. If this product were available where you shop, how likely would you be to **PURCHASE** this product?

☐ Definitely  
Would Not Buy      ☐ Probably  
Would Not Buy      ☐ Might or Might  
Not Buy      ☐ Probably  
Would Buy      ☐ Definitely  
Would Buy

xx. Overall, how well does this product meet your **EXPECTATIONS** of a **pre-packaged sandwich**?

☐ Much Worse  
Than Expected      ☐ Somewhat Worse  
Than Expected      ☐ About As  
Expected      ☐ Somewhat Better  
Than Expected      ☐ Much Better  
Than Expected

xx. Which **ONE** of the two samples do you prefer **OVERALL**?  
(Check **ONE** product code number below.)

☐ Prefer Product Seen 1st (**Code XXX**)

☐ Prefer Product Seen 2nd (**Code XXX**)

xx. What is the main reason you preferred this sample?

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**Figure 5.9** meets expectations, purchase intent and preference questions.

The responses to the closed ended questions above were converted to a numerical interpretation, averaged, and analyzed using Analysis of variance (ANOVA) with means separation (Fisher's LSD) to characterize differences among the age pair samples (SAS version 9.4, SAS Institute, Cary, NC) (Table 5.2 summarizes scales and statistical interpretation). Raw data can be found in Appendix J.18. Open ended responses ("What is the main reason you preferred this sample") were reported verbatim.



**Table 5.2** Interpretation of statistical response from the consumer test courtesy of FPI®

<b>Liking and Intensity Questions</b>
•Liking questions were based upon a 9-point scale, where 1 = dislike extremely and 9 = like extremely.
•Intensity questions were based upon a 5- or 7-point scale, with scale anchors question specific.
•For liking and intensity questions, results presented as mean scores.
•For a specific question (row), values not sharing an uppercase letter are significantly different at the 95% confidence level ( $p < 0.05$ ).
•For a specific question (row), values not sharing a lowercase letter are significantly different at the 90% confidence level ( $p < 0.1$ ).
•Rows without letters indicate no significant difference.
<b>Meets Expectations, Purchase Intent, and Preference Questions</b>
•For Meets Expectations question, analysis was run for top 2 box and bottom 2 box scores.
•For Purchase Intent, analysis was run for top 2 box scores.
•For Meets Expectations, Purchase Intent, and Preference, reported values are percentages of consumers. Values are subject to rounding error.
•Values not sharing an uppercase letter are significantly different at the 95% confidence level ( $p < 0.05$ ).
•Values not sharing a lowercase letter are significantly different at the 90% confidence level ( $p < 0.1$ ).
•Values without letters indicate no significant difference.
<b>JAR Questions</b>
•JAR Scores are based on a five-point JAR scale collapsed into three categories, where 1 = not enough, 3 = JAR, and 5 = too much of an attribute, with scale anchors question specific.
•Reported values are percentages of consumers. Values are subject to rounding error.
•For JARs 70% or greater (and less than 20% TL or TM), the attribute can be considered sufficiently optimized.
•Penalty analysis is represented only for attributes that were rated as "Too Little" (TL) or "Too Much" (TM) by at least 20% of the respondents.
•A penalty score $\geq 0.50$ is considered top penalty; $\geq 0.25$ and $< 0.50$ , middle penalty; and $< 0.25$ , bottom penalty.

The Penalty Analysis method was also used to further interpret results. Penalty analysis is used by researchers to gain insight on JAR (Just About Right) responses of the product attributes that most affect liking, purchase interest or any other product-related measure. In this study, the question “Rate the meat color of this product” was the main diagnostic question. The choice was made to phrase the responses to this question as “too light” or “too dark” as the anchor responses. This choice was made based on the belief that this concept / verbiage were more easily understood by the consumer. The alternative was to use terms like pink/red as acceptable vs. brown/grey which positioned the question as being more negative. The open ended question “What was the main reason you preferred this sample” was included to allow consumers to express comments or descriptions on meat color. Product attributes used in penalty analysis are measured with “Just -about-right” (JAR) scales. JAR scales collapse a 5 point scale to 3 point scales and helps give the researcher direction on areas of concern when 20% of the respondents rate an attribute on either side of the JAR scale (too much or not enough). When JARs results are high (~70%) of the responses as “Just About right”, this is an indication of the attribute being sufficiently optimized. Based on years of product testing, 70% can be used to indicate whether a product is fully optimized. This guideline is not correlated to

in-market performance. JAR responses are used to help determine what attributes are affecting overall liking scores and key measures. (JAR's calculation method below in Table 5.3)

**Table 5.3** JARs calculation method courtesy of FPI®

JAR calculation method.
1.Collapse 5-point Just-About-Right (JAR) scales to 3-point scales:
•4, 5 = "Too Much"
•3 = "Just-About-Right"
•1, 2 = "Not Enough"
2.Summarize distribution of JAR responses.
3.Compute average acceptance within each JAR category.
4.Compute Penalties
•Only done when $\geq 20\%$ respondents rate product not JAR
•Mean Drop = Overall Liking(JAR) - Overall Liking(Not JAR)
•"Total Penalty" = (% Not JAR)(Mean Drop)

The concept of top two and bottom two box scores was also used (abbreviated T2B and B2B). Typically ratings as interval scales are treated as equal (meaning the distance of the preference between 1 and 2 and 2 and 3 are viewed as equal distance). T2B allows the researcher to determine the percent of respondents who gave the two highest ratings (Top 2 Box). The advantage is the researcher can understand the percentage of consumers who selected the response as opposed to a ranking of 1, 2, 3, 4, 5, etc. The higher the percentage of the combination of the 1 and 2 responses, the more liked or optimized the product is on the attribute being addressed. For the sandwich / prepackaged food category, a T2B box score of 70% is a good indication that the product likely to be purchased, while 20% for a B2B is an indication that the product is not likely to be purchased.

### **5.1.3 Overview of the Oxygen (O<sub>2</sub>) phase from manufacture to refrigerated shelf life**

To review, the percent O<sub>2</sub> in the headspace of the sandwich package varies from post-manufacture to sandwich consumption. The variation can be described in three phases. The first phase is the O<sub>2</sub> level immediately following vacuum, gas flush, and seal. The second phase occurs minutes after sealing through frozen storage which can last from 3 days to 9 months (product is held a minimum of 3 days before distribution to ensure complete freeze). The third and final phase is the start of refrigerated shelf life at retail display until consumption (1 to 30 days after refrigeration).

For this test, the O<sub>2</sub> percentage in the headspace of the packages were measured for the wedge format sandwiches both during immediate frozen storage (phase 2) and during the 30 day refrigerated shelf life (phase 3). The flat format sandwiches (used in the  $L*a*b^*$  color measurement) were measured in phase 3 only. A few sandwiches were checked in phase 1 to ensure that the desired O<sub>2</sub> levels were being achieved at the time of manufacture (< 0.5%). During the production day of the sample run, Quality Assurance recorded initial levels ranging from ppm to 0.21% in phase 1 for both Multivac machines used (one for the meat shape and one for the wedge shape) (Appendix J.10 and J.11).

#### **5.1.3a Residual oxygen levels in the headspace during phase 2 (frozen storage) – Test 10**

Tracking O<sub>2</sub> levels for forty hours following sandwich packaging was done for a better understanding of changes during phase 2. A key factor to establish is the amount of trapped air released in this stage. A single wedge format sandwich was pulled from the freezer for each treatment, checked with a Mocon and discarded. After 16 hours in the freezer, both treatments saw an increase in O<sub>2</sub> levels with the control sample peaking at 1.35% in a single package, while the sachet packaged sandwich peaked at 0.303 % in the same time frame (Table 5.4).

**Table 5.4** Raw data oxygen and carbon dioxide percentage in the packaging headspace during the first 40 hours of frozen storage for a single package

Scavenger sachet			Control (no scavenger)		
time					
(hours)	CO <sub>2</sub> %	O <sub>2</sub> %	time (hours)	CO <sub>2</sub> %	O <sub>2</sub> %
0	23.5	0.003	0	23.6	0.034
6	21.5	0.059	6	22.5	0.033
16	22.3	0.303	16	22.2	1.35
22	21.5	0.161	22	23.5	0.992
30	23.6	0.073	30	24.2	0.202
40	23.9	0.099	40	25.1	0.197

Oxygen levels for both treatments increased at 16 hours, then decreased to levels below 0.2% after 40 hours. For the sachet, the O<sub>2</sub> level was generally below the control except at 6 hours. This result indicates that the sachet was somewhat effective in minimizing the headspace O<sub>2</sub> by absorbing trapped oxygen faster than other competing reactions.

Two follow up tests were conducted on separate batches of control ham and cheese wedges in the retail wedge format to increase the sample size and verify the results of the above finding. Full cases of sandwiches selected at random were checked immediately following sealing of the package, and at three time intervals during the freeze down process (at hours 0, 23, 27 and 29.5 in the first repeat test and 0, 5, 20 and 24 for the second). Both tests confirmed that at least one sandwich in the case had an oxygen level greater than 1.0% (Table 5.5 -5.6).

**Table 5.5** Follow up test 1 for oxygen and carbon dioxide levels in the headspace 30 hours after freezing. Yellow and red highlighted numbers indicate packages with higher O<sub>2</sub> levels comparatively to other products in the case

5/4/2015 9:42:00 AM Immediately following production (time = 0)			8:40 am on 5/5/15 (hour 23)		
<b>Case 1</b>	<b>CO2</b>	<b>O2</b>	<b>Case 2</b>	<b>CO2</b>	<b>O2</b>
sandwich 1	21.00	0.1120	sandwich 1	18.70	0.3690
sandwich 2	21.00	0.0943	sandwich 2	18.20	1.6664
sandwich 3	20.70	0.1830	sandwich 3	19.00	0.1300
sandwich 4	20.50	0.0282	sandwich 4	18.90	0.1550
sandwich 5	21.00	0.0000	sandwich 5	18.80	0.1110
sandwich 6	20.50	0.0000	sandwich 6	19.10	0.1930
sandwich 7	20.30	0.0113	sandwich 7	19.10	0.1210
sandwich 8	20.40	0.0000	sandwich 8	19.20	0.1790
sandwich 9	20.40	0.0000	sandwich 9	18.70	0.1100
sandwich 10	20.20	0.0004	sandwich 10	leaker	
<i>min</i>	20.20	0.0000	<i>min</i>	18.20	0.1100
<i>max</i>	21.00	0.1830	<i>max</i>	19.20	1.6664
<i>range (max-m</i>	0.80	0.1830	<i>range (max-min)</i>	1.00	1.5564
12:40 pm on 5/5/15 (hour 27)			3:00 pm on 5/5/15 (hour 29.5)		
<b>Case 1</b>	<b>CO2</b>	<b>O2</b>	<b>Case 1</b>	<b>CO2</b>	<b>O2</b>
sandwich 1	18.50	0.1170	sandwich 1	18.90	0.1020
sandwich 2	19.80	0.0900	sandwich 2	19.30	0.1270
sandwich 3	20.20	0.1190	sandwich 3	19.70	0.1030
sandwich 4	19.10	0.1160	sandwich 4	19.60	0.1240
sandwich 5	19.10	0.0940	sandwich 5	19.20	0.1280
sandwich 6	19.00	0.1000	sandwich 6	18.90	0.1410
sandwich 7	19.30	0.0880	sandwich 7	17.90	0.1680
sandwich 8	19.30	0.1280	sandwich 8	18.50	0.1590
sandwich 9	19.10	0.0740	sandwich 9	19.40	0.0990
sandwich 10	19.20	0.1210	sandwich 10	19.40	0.0900
<i>min</i>	18.50	0.0740	<i>min</i>	17.90	0.0900
<i>max</i>	20.20	0.1280	<i>max</i>	19.70	0.1680
<i>range (max-m</i>	1.70	0.0540	<i>range (max-min)</i>	1.80	0.0780

**Table 5.6** Follow up test 2 for oxygen and carbon dioxide levels in the headspace 24 hours after freezing. Yellow and red highlighted numbers indicate packages with higher O<sub>2</sub> levels comparatively to other products in the case

MOCON Check #1 - 2122	7/28/2015		11:00 AM	MOCON C	7/29/2015		7:00 AM
0 hour	CO2 %	O2 %		20 hour	CO2 %	O2 %	
1	21.4	0.0528		1	21.2	0.21	
2	21	0.0766		2	21.3	0.181	
3	20.6	0		3	21.2	0.131	
4	21.1	0		4	21.8	0.142	
5	20.6	0		5	19.8	1.24	
6	20.6	0		6	21	0.173	
7	20.2	0.0022		7	21.3	0.113	
8	20.9	0		8	21.4	0.112	
9	21.3	0.0252		9	21	0.117	
10	20.7	0		10	21.1	0.122	
Max.	21.400	0.077		Max.	21.800	1.240	
Min.	20.200	0.000		Min.	19.800	0.112	
Range	1.200	0.077		Range	2.000	1.128	
MOCON Check #2	7/28/2015		4:00 PM	MOCON C	7/29/2015		11:00 AM
5 hour	CO2 %	O2 %		24 hour	CO2 %	O2 %	
1	19.6	0.161		1	21.2	0.184	
2	19.5	0.128		2	21.1	0.173	
3	20.2	0.154		3	21	0.159	
4	19.2	0.144		4	21.1	0.131	
5	19.3	0.113		5	20.8	0.276	
6	19.5	0.128		6	20.9	0.127	
7	19.0	0.111		7	20.8	0.163	
8	19.6	0.124		8	20.9	0.157	
9	19.0	0.115		9	21.3	0.162	
10	19.0	0.136		10	21.1	0.103	
Max.	20.200	0.161		Max.	21.300	0.276	
Min.	19.000	0.111		Min.	20.800	0.103	
Range	1.200	0.050		Range	0.500	0.173	

All three tests show that a peak oxygen level occurs between 16 – 23 hours post packaging, with at least one sandwich per case above 1.0% O<sub>2</sub>. As previously reviewed, the design of the evacuation chamber for the Multivac is such that the middle cavities do not see the same strength of vacuum as the outer cavities. A likely explanation for the above outcome is that within each case (which typically represents sandwiches made in 1 cycle of the MAP equipment); there are several sandwiches that have higher oxygen in the headspace as a result of the middle cavities of the evacuation chamber design receiving less vacuum during evacuation.

If the two higher O<sub>2</sub> values are discarded from hour 0 and 23, the average O<sub>2</sub> difference between hours 0 and 23 is 0.125% (0.1427% - 0.0168%, Table 5.4). The result of both phase 2 tests supports that oxygen levels increase to varying degrees immediately following phase 1 manufacturing and sealing, but decrease after 24 hours to levels that are 0.1 to 0.2% higher than the levels immediately following manufacture.

Although average values are not as useful because of the high variability between packages, it is helpful to understand the average increase that occurs on the majority of the sandwiches from phase 1 to 2, which can help in the calculation of the amount of oxygen needed to be removed. The Carbon dioxide level also decreases on average immediately following production. This supports CO<sub>2</sub> dissolving into the sandwich to form carbonic acid, which has the potential to lower surface pH, which is a benefit as a microbial hurdle to growth over time.

Potential explanations for the changes in headspace oxygen during the first 24 hours post-sealing of the package (not including the initial production O<sub>2</sub> variation introduced as is described in Test 2) include trapped air between sandwich components diffusing into the headspace, followed by microbial activity and absorption back into the ham, cheese and bread (Via multiple potential reactions including lipid oxidation, thermal oxidation, enzymatic activity, and antioxidant activity). While the potential exists for O<sub>2</sub> to be consumed by aerobic microorganisms and enzymatic activity after packaging, the freezer temperature decreases activity for both. It is more likely that the oxygen is being reabsorbed by the bread, cheese and meat through other reactions. For meat pigments there is the potential for ionic attractions and oxidation/reduction reactions occurring that are setting up intermediate forms that will later contribute to further oxidation / reduction of the meat pigments as the warmer temperatures and light are introduced during the sandwich thaw. As previously stated, the mechanism of meat discoloration is complex, has many unobserved intermediate forms, and is not completely understood. (Johnston, Knight and Ledward, 1992, Møller and Skibsted, 2002). While light serves as a catalyst, it is not known if the complete reaction initiates once light is introduced, or if intermediates are formed during dark storage, and then proceeds to completion in the light. Complete removal of O<sub>2</sub> as quickly as possible is important as oxygen is a key element responsible for food deterioration.

Møller et al. also found increases in O<sub>2</sub> headspace percentages in sliced ham only after one day of refrigerated storage which was attributed to trapped air in the ham slices. (Møller et al., 2000). A key difference with sandwiches from sliced meat alone is the potential of trapped air in bread which can vary significantly from package to package

(the sandwich bread in this study has a slice weight variance of 0.9 to 1.3 oz.). With white bread having a porosity of 64.4 – 84 % with 99% of the pores connected (Wang, 2014); each sandwich package is more unique for gas composition and results in greater variability from package to package.

It is also possible that lipid oxidation can occur in the ham, but lipid oxidation (which can be measured using the TBARS method) and sensory odor has not been detected in multiple studies of sliced ham packaged in MAP with oxygen scavengers (likely attributed to the low unsaturated fat percentage in ham) (Chaiyapechara, Meng, and Hotchkiss, 1998; Anderson and Rasmussen, 1992; Haile et al., 2013). It is possible for a sandwich, that pasteurized processed cheese is responsible for some O<sub>2</sub> consumption via lipid oxidation, although that generally has an unsaturated fat level at < 18%.

### **5.1.3b Residual O<sub>2</sub> levels during phase 3 (refrigerated shelf life) consumer wedge shape**

For the consumer wedge format sample sandwiches, the gas levels in the headspace were checked one day after the consumer study. The outcome for all age pairs of product was 0% oxygen was attained in both the control and scavenger samples (Table 5.7).



**Table 5.7** Gas levels in the headspace of wedge format sandwiches used in the consumer study

Consumer code	description	CO <sub>2</sub> %	O <sub>2</sub> %	age (day of check)
397	control package	21.0	0.0000	5
397	control package	20.0	0.0000	5
397	control package	20.1	0.0000	5
397	control package	19.9	0.0000	5
840	prototype package	18.6	0.0000	5
840	prototype package	16.3	0.0000	5
840	prototype package	17.3	0.0000	5
840	prototype package	17.0	0.0000	5
840	prototype package	16.6	0.0000	5
559	prototype package	18.4	0.0000	8
559	prototype package	17.2	0.0000	8
559	prototype package	16.1	0.0000	8
559	prototype package	14.0	0.0000	8
559	prototype package	15.2	0.0000	8
168	control package	20.4	0.0000	8
168	control package	19.8	0.0000	8
168	control package	19.9	0.0000	8
168	control package	19.3	0.0000	8
426	prototype package	14.4	0.0000	31
426	prototype package	13.6	0.0000	31
426	prototype package	13.7	0.0000	31
426	prototype package	15.5	0.0000	31
783	control package	20.8	0.0000	31
783	control package	18.0	0.0000	31
783	control package	17.9	0.0000	31
783	control package	19.6	0.0000	31
783	control package	18.2	0.0000	31

This outcome is consistent with some of the previous tests. Multiple possibilities exist for why all O<sub>2</sub> readings were zero for the consumer reviewed wedge format sandwiches including 1) Any residual oxygen present at the start of the refrigerated shelf life was consumed from thaw to day 4; 2) Initial O<sub>2</sub> percentages at phase 1 were low; 3) Any initial residual oxygen was consumed during phase 2 (frozen storage), or 4) Instrumental error on the Mocon (Section 3.12 – Mocon Pac Check® is accurate up to +/- 0.05% for the absolute reading). For the sachet, the lower O<sub>2</sub> levels are likely the result of the sachet scavenging available oxygen.

### 5.1.3c Residual O<sub>2</sub> levels during phase 3 – Flat face (square format)

For the flat format sandwiches used in the color analysis, the scavenger packaged sandwiches started and remained at 0% oxygen in the headspace at all points during the

30 day refrigerated shelf life. The control samples ranged from 0 to 0.155% through day 7, but proceeded to 0% oxygen in the headspace by day 14 through day 30 (Table 5.8).

**Table 5.8** Gas levels in the headspace of flat format sandwiches used in  $L^*$  and  $a^*$  analysis in test 10

day	Control	Control	Control	Multisorb	Multisorb	Multisorb
	lane A	lane B	lane C	lane A	lane B	lane C
	oxygen %	oxygen %	oxygen %	oxygen %	oxygen %	oxygen %
1	0.008	0.051	0.078	0.000	0.000	0.000
4	0.000	0.001	0.018	0.000	0.000	0.000
7	0.003	0.053	0.155	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000	0.000
30	0.000	0.000	0.000	0.000	0.000	0.000
<b>min</b>	0.000	0.000	0.000	0.000	0.000	0.000
<b>max</b>	0.008	0.053	0.155	0.000	0.000	0.000
<b>range</b>	0.008	0.053	0.155	0.000	0.000	0.000

The difference in oxygen percentage in the headspace between the control flat format sandwiches compared to wedge format in this study can be explained by 1) wedges and the flats were produced the same day, but on a separate Multivac equipment (each will have different starting variability) and 2) greater product to headspace ratio creates more gas to be evacuated (Greater void space in the package creates more work for the vacuum).

O<sub>2</sub> differences between the control and scavenger packages in the flat format can also be explained as follows. Typically white bread has a porosity of 64.4 – 84 % with 99% of the pores connected. (Wang, 2014) Thus the vacuum packaging process (3 seconds) lowers the O<sub>2</sub> level in the air space in the package creating a driving force that causes the O<sub>2</sub> level to increase in the headspace post-sealing as the gas flows out of the bread pores.

Then the sandwich components including the meat pigment reacts with the available O<sub>2</sub> and the level decreases to zero over time. The scavenger sachet system however supposedly reacts fast enough with the oxygen to keep it at zero over the length of the shelf life. Because the bread slices varies in size for each individual sandwich, the resulting trapped air varies from package to package.

As proven many times in tests 1 – 10, the amount of residual oxygen available varies significantly from package to package. This is an observation worth noting as this is a variable that could explain why apparently random sandwiches within a cooler set will show significant discoloration when others around it do not. The lane C control flat in this test had more residual oxygen per package than lanes A or B. While this is potentially just package to package variation, it also could be explained by more oxygen being consumed in lanes A and B in the meat discoloration reaction.

#### **5.1.4 Ham *a*\* color analysis Test 10**

Color analysis was conducted on the flat ham packages. All products were removed from the package and analyzed for CIE *L*\* and *a*\*, for Illuminant C, aperture of 50 mm and standard observe angle of 2° using a Konica Minolta Chroma Meter CR-410 (Minolta, Osaka, Japan). Method 2 for preparing the surface measurement (flat ham measurement) was used (as outlined in Methods & Materials Figure 3.10) and consistent with the method used in Tests 6-9. As seen in previous tests, the variability of *a*\* values within and across all lanes over time again was large for both treatments with a range of scores from 15.65 to 18.82 for the ferrous based scavenger sachet and 9.32 to 18.82 for the control package (Table 5.9).

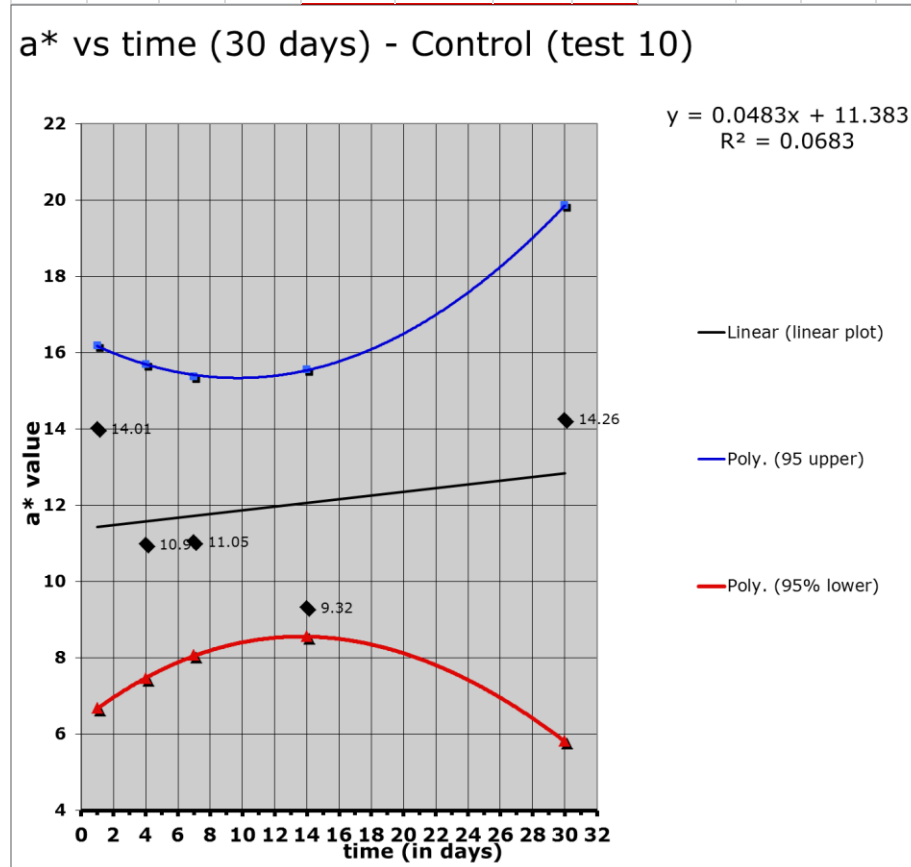
**Table 5.9** Difference in  $a^*$  of scavenger compared to control (flat ham) at each age pair  
Test 10 (For lanes A, B, and C)

LANE A day	Control lane A $a^*$	Control lane B $a^*$	Control lane C $a^*$	Multisorb lane A $a^*$	Multisorb lane B $a^*$	Multisorb lane C $a^*$
1	14.01	18.82	17.10	17.19	18.82	17.60
4	10.98	13.99	13.09	18.26	17.33	15.65
7	11.05	15.23	14.87	16.25	17.46	16.63
14	9.32	11.39	14.20	17.01	17.99	16.31
30	14.26	11.03	14.13	17.80	17.02	17.71
min	9.32	11.03	13.09	16.25	17.02	15.65
max	14.26	18.82	17.10	18.26	18.82	17.71
range	4.94	7.80	4.01	2.01	1.80	2.06

Entering the  $a^*$  values from Table 5.9 above into the kinetics data input sheet (Tables 5.10 – 5.15) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

**Table 5.10** Test 10  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the control package in Lane A

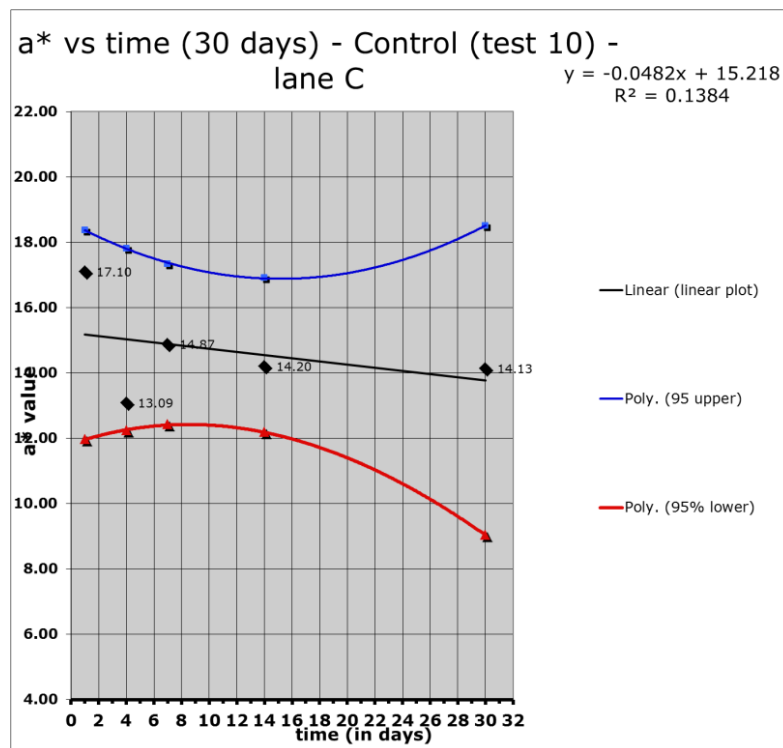
2. Calculation Note after entering Y and X you need to pull down formulas in each column from top to last entry															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
14.01	1.0	196.28	14.01	11.43	1.00	14.01	11.43	6.65	104.04	14.01	1.00	16.19	6.68	9.51	11.43
10.98	4.0	120.56	10.98	11.58	16.00	10.98	11.58	0.36	51.84	43.92	16.00	15.70	7.45	8.25	11.58
11.05	7.0	122.10	11.05	11.72	49.00	11.05	11.72	0.45	17.64	77.35	49.00	15.37	8.07	7.31	11.72
9.32	14.0	86.86	9.32	12.06	196.00	9.32	12.06	7.50	7.84	130.48	196.00	15.57	8.55	7.01	12.06
14.26	30.0	203.35	14.26	12.83	900.00	14.26	12.83	2.04	353.44	427.80	900.00	19.85	5.81	14.05	12.83
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average
slope=												Standard Error			
intercept=												Sum (yi-yes)			
rsq=												n			
± 95% slope												t 95%,2,n-2=			
k upper												x average =			
k lower															
Equations												Sum (xi-xav)			
Y = 11.3834      0.0483      * time												(Sum x)^2			
												Sum(y^2)			
												sum y			
												Sum (xi*Yi)			
												sum x			
												sum (X^2)			



**Figure 5.10 A:** Control sample test 10 Zero order Linear plot of  $a^*$  versus time (30 days) with 95% confidence limits calculation

**Table 5.11** Test 10  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the control package in Lane B

1. Raw Data:																
# data pairs Total=		5		This is automatically counted												
Y units		a*		control lane B												
X units		days														
STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry (cyl-yes)^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate		(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte
	18.82	1.0	354.32	18.82	16.33	1.00	18.82	16.33	6.21	104.04	18.82	1.00	20.74	11.93	8.81	16.33
	13.99	4.0	195.72	13.99	15.67	16.00	13.99	15.67	2.83	51.84	55.96	16.00	19.49	11.85	7.64	15.67
	15.23	7.0	231.85	15.23	15.01	49.00	15.23	15.01	0.05	17.64	106.59	49.00	18.40	11.63	6.77	15.01
	11.39	14.0	129.66	11.39	13.48	196.00	11.39	13.48	4.36	7.84	159.41	196.00	16.72	10.23	6.50	13.48
	11.03	30.0	121.59	11.03	9.96	900.00	11.03	9.96	1.14	353.44	330.80	900.00	16.47	3.45	13.01	9.96
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte
															Standard Error	2.20
															Sum (yi-yes)	1658.25
															n	5.00
															t 95%, 2, n-2=	3.18
															x average =	11.20
															Sum (xi-xav	1287.44
															(Sum x)^2	3136.00
															Sum(y^2)	1033.13
															sum y	70.45
															Sum (xi*yi)	671.58
															sum x	56.00
															sum (X^2)	1162.00

[illegible]

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1. Raw Data:

# data pairs

Total=

5

This is automatically counted

Y units

a\*

Multisorb lane A

X units

days

2. Calculations

Note after entering Y and X you need to pull down formulas in each column from top to last entry

(y<sub>i</sub>-y<sub>est</sub>)<sup>2</sup>

(x<sub>i</sub>-x<sub>ave</sub>)<sup>2</sup>

x<sub>i</sub>\*y<sub>i</sub>

X<sup>2</sup>

y 95%U.L

y 95%L.L

Delta

predicted average

Y value	x= time	Y <sup>2</sup>	Y plot value	Est y <sub>i</sub>	time <sup>2</sup>	y <sub>i</sub>	y <sub>i</sub> estimate	(y <sub>i</sub> -y <sub>est</sub> ) <sup>2</sup>	(x <sub>i</sub> -x <sub>ave</sub> ) <sup>2</sup>	X <sub>i</sub> *Y <sub>i</sub>	X <sup>2</sup>	y 95%U.L	y 95%L.L	Delta	predicted average
17.19	1.0	295.50	17.19	17.16	1.00	17.19	17.16	0.00	104.04	17.19	1.00	18.90	15.43	3.48	17.16
18.26	4.0	333.43	18.26	17.20	16.00	18.26	17.20	1.11	51.84	73.04	16.00	18.71	15.70	3.02	17.20
16.25	7.0	264.06	16.25	17.25	49.00	16.25	17.25	0.99	17.64	113.75	49.00	18.58	15.91	2.67	17.25
17.01	14.0	289.34	17.01	17.34	196.00	17.01	17.34	0.11	7.84	238.14	196.00	18.62	16.06	2.56	17.34
17.8	30.0	316.84	17.80	17.56	900.00	17.80	17.56	0.06	353.44	534.00	900.00	20.12	14.99	5.14	17.56
Y value	x= time	Y <sup>2</sup>	Y plot value	Est y <sub>i</sub>	time <sup>2</sup>	y <sub>i</sub>	y <sub>i</sub> estimate	(y <sub>i</sub> -y <sub>est</sub> ) <sup>2</sup>	(x <sub>i</sub> -x <sub>ave</sub> ) <sup>2</sup>	X <sub>i</sub> *Y <sub>i</sub>	X <sup>2</sup>	y 95%U.L	y 95%L.L	Delta	predicted average

slope=

0.0135

intercept=

17.1510

rsq=

0.0410

± 95% slope

0.1197

k upper

0.1332

k lower

-0.1062

Standard Error

0.87

Sum (y<sub>i</sub>-y<sub>est</sub>)<sup>2</sup>

1767.22

n

5.00

t 95%,2,n-2

3.18

x average =

11.20

Sum (x<sub>i</sub>-x<sub>ave</sub>)<sup>2</sup>

1287.44

(Sum x)<sup>2</sup>

3136.00

Sum (y<sup>2</sup>)

1499.17

sum y

86.51

Sum (x<sub>i</sub>\*y<sub>i</sub>)

976.12

sum x

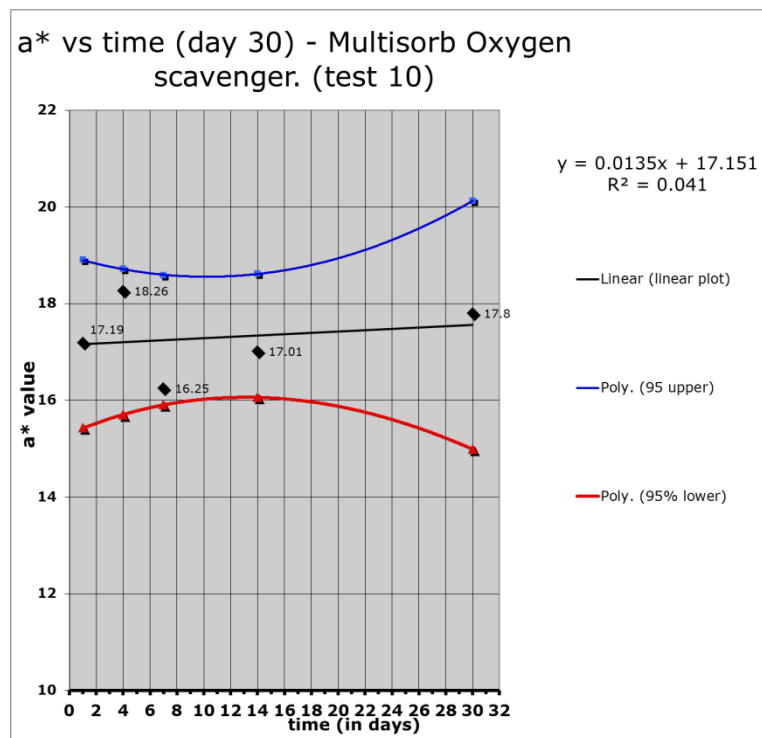
56.00

sum (X<sup>2</sup>)

1162.00

Equations

Y = 17.1510 + 0.0135 \* time

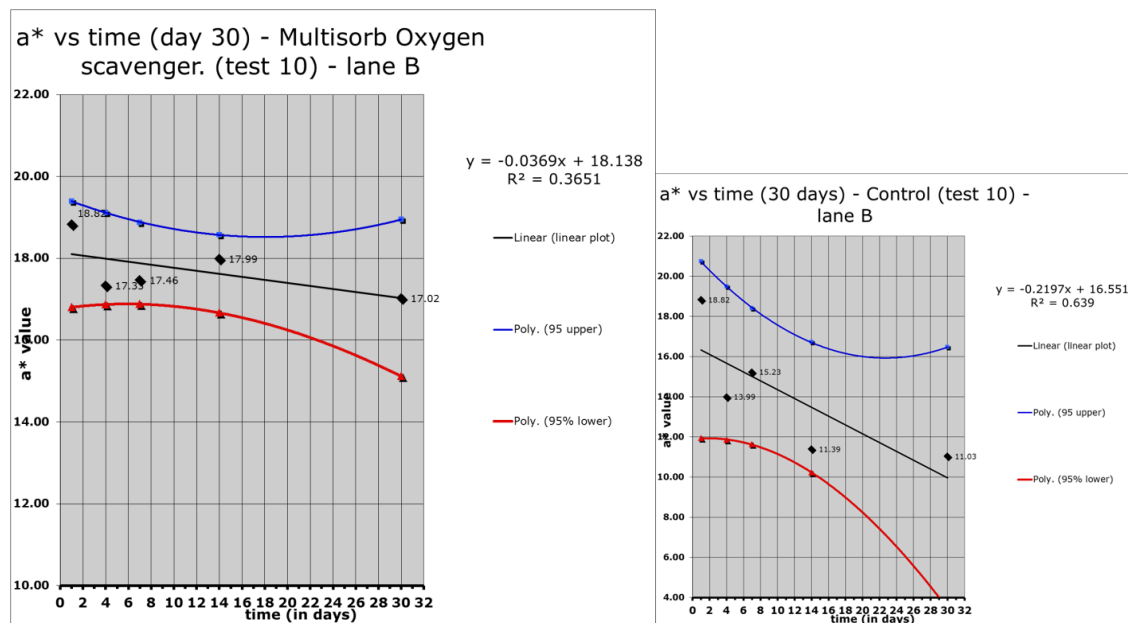


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**Table 5.14** Test 10  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the O<sub>2</sub> scavenger sachet package in Lane B

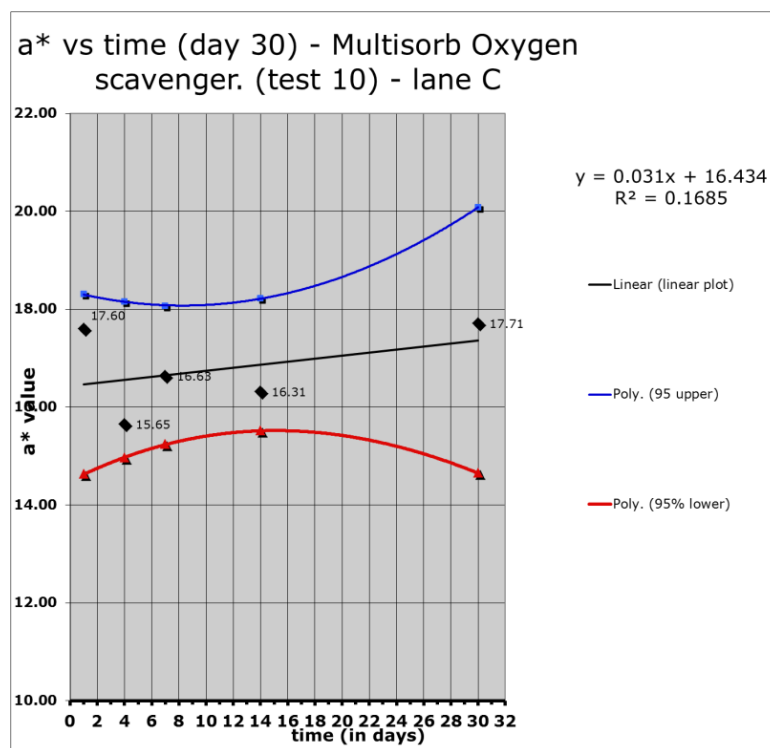
1. Raw Data:																
# data pairs Total=		5 This is automatically counted														
Y units		a* Multisorb lane B														
X units		days														
STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry r(yi-yes)^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate		(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	18.82	1.0	354.32	18.82	18.10	1.00	18.82	18.10	0.52	104.04	18.82	1.00	19.40	16.80	2.59	18.10
	17.33	4.0	300.33	17.33	17.99	16.00	17.33	17.99	0.44	51.84	69.32	16.00	19.12	16.87	2.25	17.99
	17.46	7.0	304.97	17.46	17.88	49.00	17.46	17.88	0.17	17.64	122.24	49.00	18.88	16.88	1.99	17.88
	17.99	14.0	323.52	17.99	17.62	196.00	17.99	17.62	0.13	7.84	251.81	196.00	18.58	16.67	1.91	17.62
	17.02	30.0	289.79	17.02	17.03	900.00	17.02	17.03	0.00	353.44	510.70	900.00	18.95	15.12	3.83	17.03
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	Xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicte average
	slope= -0.0369												Standard Er	0.65		
	intercept= 18.1383												Sum (yi-yes	1975.25		
	rsq= 0.3651												n	5.00		
	± 95% slope 0.0893												t 95%,2,n-2=	3.18		
	k upper 0.0524												x average =	11.20		
	k lower -0.1261															
	Equations												Sum (xi-xav	1287.44		
	Y = 18.1383 -0.0369 * time												(Sum x)^2	3136.00		
													Sum(y^2)	1572.93		
													sum y	88.63		
													Sum (xi*yi)	972.90		
													sum x	56.00		
													sum (X^2)	1162.00		



**Figure 5.14** Lane B: Scavenger system Test 10 Zero order plot of  $a^*$  versus time (30 days) with 95% confidence limits calculation.

**Table 5.15** Test 10  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the O<sub>2</sub> scavenger sachet package in Lane C

1. Raw Data:																
# data pairs Total=		5 This is automatically counted														
Y units		a* Multisorb lane C														
X units		days														
STATISTICS																
2. CalculatiNote after entering Y and X you need to pull down formulas in each column from top to last entry (y1-yes)^2																
	Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	y1 estimate		(x1-xave)^2	x1*y1	X^2	y 95%UL	y 95%LL	Delta	predicte
	17.60	1.0	309.76	17.60	16.47	1.00	17.60	16.47	1.29	104.04	17.60	1.00	18.30	14.63	3.68	16.47
	15.65	4.0	245.03	15.65	16.56	16.00	15.65	16.56	0.82	51.84	62.61	16.00	18.15	14.96	3.19	16.56
	16.63	7.0	276.67	16.63	16.65	49.00	16.63	16.65	0.00	17.64	116.43	49.00	18.06	15.24	2.82	16.65
	16.31	14.0	266.02	16.31	16.87	196.00	16.31	16.87	0.31	7.84	228.34	196.00	18.22	15.51	2.71	16.87
	17.71	30.0	313.76	17.71	17.37	900.00	17.71	17.37	0.12	353.44	531.40	900.00	20.08	14.65	5.43	17.37
	Y value	x=time	Y^2	Y plot value	Est y1	time^2	y1	y1 estimate	(y1-yes)^2	(x1-xave)^2	X1*Y1	X^2	y 95%UL	y 95%LL	Delta	predicte
															Standard Error	0.92
															Sum (y1-yes)	1623.09
															n	5.00
															t 95%, 2, n-2	3.18
															x average =	11.20
															Sum (x1-xav)	1287.44
															(Sum x)^2	3136.00
															Sum(y^2)	1411.23
															sum y	83.91
															Sum (x1*y1)	956.39
															sum x	56.00
															sum (X^2)	1162.00
</																

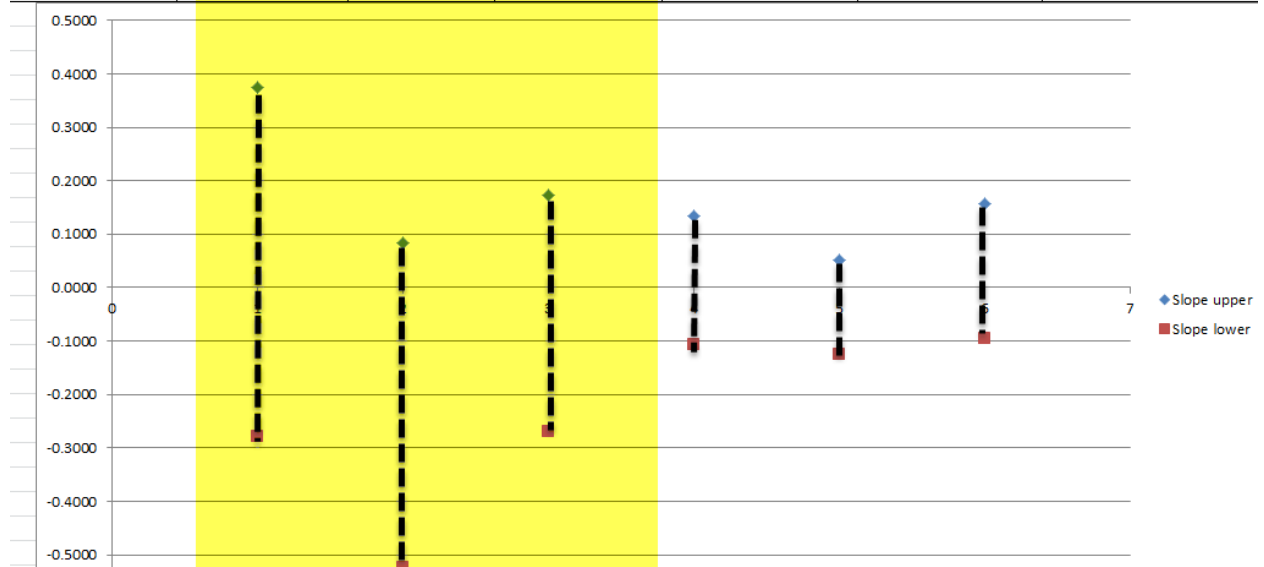


**Figure 5.15** Lane C: Scavenger system test 10 Zero order plot of  $a^*$  versus time (30 days) with 95% confidence limits calculation

Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant (k) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant (k) overlap between treatments is provided in Table 5.16. Any overlap in rate constant (k) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 5.16**  $a^*$  rate constant (k) upper and lower for both treatments in Test 10 lanes A-C as established by Labuza' Reaction kinetics shelf life model

$a^*$ slopes Test 10	current package Control lane A	current package Control lane B	current package Control lane C	Multisorb® ferrous scavenger sachet lane A	Multisorb® ferrous scavenger sachet lane B	Multisorb® ferrous scavenger sachet lane C
Slope upper	0.3756	0.0835	0.1726	0.1332	0.0524	0.1576
Slope lower	-0.2790	-0.5229	-0.2690	-0.1062	-0.1261	-0.0955
Slope	0.0483	-0.2197	-0.0482	0.0135	-0.0369	0.0310
95% CL +/-	0.3273	0.3032	0.2208	0.1197	0.0893	0.1265
R2	0.0683	0.6390	0.1384	0.0410	0.3661	0.1685



Viewed over 30 days for all lanes, the control predicted  $a^*$  value slopes are not statistically different compared to the scavenger treatment, however the scavenger packaged treatments have a narrower range of outcomes in all lanes (greater predictability) compared to the control.

The trend line slope of the line for the scavenger treatment is positive (increasing redness) in lanes A and C, while the control is negative (decreasing redness) in lanes B and C. The slope of the line in lane A comparing the scavenger and control predicts a  $\Delta a^* \geq 4$  by day 0 of the shelf life with the y intercept for the scavenger = 17.15 compared to 11.38 for the control package (Table 5.10, 5.13). This distance remains comparable throughout the 30 day study. In lane B, the model predicts a  $\Delta a^* \geq 4$  between treatments by day 14 (Table 5.11, 5.14). In lane C, the model predicts a  $\Delta a^* \geq 4$  between treatments by day 30 (Table 5.12, 5.15).

As previously established,  $\Delta a^* \geq 4$  correlates to a visual loss in redness to the naked eye (Anderson and Rasmussen, 1992). Anderson and Rasmussen completed a similar evaluation with sliced ham using a 50 cc oxygen scavenging sachet. Comparing sliced ham in a vacuum package to sliced ham stored with an oxygen scavenger and also measuring with a tristimulus colorimeter, they reported a  $\Delta a = 4$  at 16 hours between the two treatments which corresponded to a significant change in appearance as observed by a color panel (where a  $\Delta a < 1$  was a negligible difference in color as reported by the color panel). The scavenger treatment had a higher  $a$  value score compared to the control (non-scavenger) at all checkpoints throughout the 24 day study (Anderson and Rasmussen, 1992)

In our study  $a^*$  value color score for the scavenger started and ended with about the same approximate value (Lane A day 1  $a^* = 17.19$ , day 30  $a^* = 17.8$ ) The same phenomenon was observed with the control (Lane A day 1  $a^* = 14.01$  and at day 30  $a^* = 14.26$ ). Thus  $a^*$  scores for both control and scavenger could be interpreted as not changing very much over time. However, the improvement in  $a^*$  value at the end of the shelf life could be attributed to potential moisture loss in the ham, given the differences in the water activities between the meat ( $\sim 0.97$ ), cheese ( $\sim 0.95$ ), and the bread ( $\sim 0.91$ ). Under these conditions the meat will lose moisture to the cheese and bread (Bell and Labuza, 1994) and thus drying out changed the visual appearance of the ham which results in a more concentrated meat pigments and intense color. Anderson and Rasmussen also found that the red color of refrigerated ham when packaged separately partially reestablishes color after an initial decrease, suggesting other dynamics in the packaging favoring an

equilibrium for nitrosylhemochrome development that results in improve color score over time (Anderson and Rasmussen, 1992). Another explanation for a trend towards improved color over time is the change in the pH of the cured ham over the course of the shelf life. Using wedge format sandwiches from the consumer study, the pH was measured for a single sandwich at days 4 and 30 and confirmed the meat pH value decreases over time (Table 5.17).

**Table 5.17** pH of each sandwich component part at day 4 and day 30 of refrigerated storage in the display cabinet

component	day 4	day 30
	pH	pH
Bread	5.7	5.82
Meat	6.21	5.87
Cheese	6.1	5.86

Given the small initial sample size, a follow up test was set up to measure the pH in triplicate at days 1, 7, 14 and 25. The results of this evaluation demonstrates that pH does change over time (Ham starting average pH of 6 which decrease to 5.89 by day 14) , however there is variability from day to day which provides another changing condition that may explain variation from package to package (Table 5.18).

**Table 5.18** follow up pH check of each sandwich component part at multiple points of refrigerated storage

6/17/2015	Day 1:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	5.88	5.96	5.42
	<b>2</b>	6.07	6.03	5.32
	<b>3</b>	5.96	6.01	5.40
	average	5.97	6.00	5.38
	min	5.88	5.96	5.32
	max	6.07	6.03	5.42
6/25/2015	Day 7:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	6.19	6.00	5.71
	<b>2</b>	6.21	5.94	5.72
	<b>3</b>	6.17	5.97	5.74
	average	6.19	5.97	5.72
	min	6.17	5.94	5.71
	max	6.21	6.00	5.74
7/1/2015	Day 14:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	6.02	5.87	5.60
	<b>2</b>	5.98	5.90	5.65
	<b>3</b>	6.06	5.91	5.70
	average	6.01	5.89	5.65
	min	5.98	5.87	5.65
	max	6.06	5.91	5.70
7/15/2015	Day 28:	(pH)		
		<b>Cheese</b>	<b>Ham</b>	<b>Bread</b>
	<b>1</b>	6.17	6.03	5.73
	<b>2</b>	6.18	5.98	5.81
	<b>3</b>	6.15	5.95	5.82
	average	6.17	5.99	5.79
	min	6.15	5.95	5.73
	max	6.18	6.03	5.82

The decreasing pH at day 30 may promote reduction of nitrite derivatives which in the presence of ascorbate ions drives favorable reformation reactions for desired pigment. Anderson and Rasmussen also observed pH values decreasing over time with sliced ham packaged separately. When packaged with the oxygen scavenger, they found discoloration to be eliminated in sliced ham. (Anderson and Rasmussen, 1992)

### 5.1.5 – $L^*$ scores Test 10

The variability of  $L^*$  values across both treatments over time was large for both treatments with a range of scores for the control across all lanes from 56.52 to 64.18 ( $\Delta L^* = 7.66$ ) and a range of 53.86 to 61.92 ( $\Delta L^* = 8.06$ ) for the ferrous scavenger sachet (Table 5.19).

**Table 5.19**  $L^*$  values over time for control and scavenger in all lanes Test 10

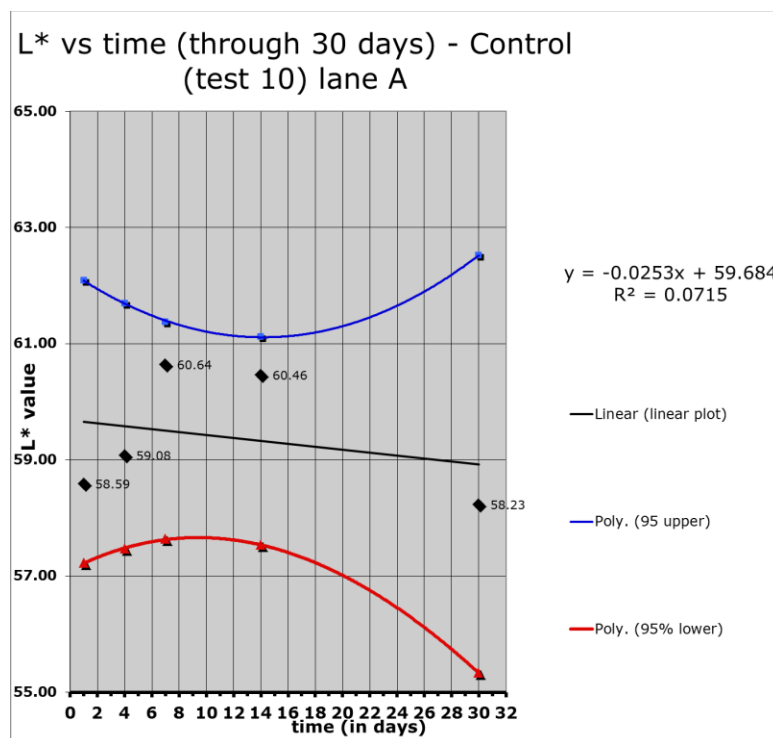
day	Control lane A $L^*$	Control lane B $L^*$	Control lane C $L^*$	Multisorb lane A $L^*$	Multisorb lane B $L^*$	Multisorb lane C $L^*$
1	58.59	56.52	57.50	60.95	57.18	60.63
4	59.08	60.16	64.18	57.86	59.94	61.92
7	60.64	59.79	60.37	61.01	58.90	60.70
14	60.46	58.91	62.16	58.84	57.37	59.61
30	58.23	59.50	58.46	53.86	59.31	60.46
<b>min</b>	58.23	56.52	57.50	53.86	57.18	59.61
<b>max</b>	60.64	60.16	64.18	61.01	59.94	61.92
<b>range</b>	2.41	3.64	6.68	7.16	2.76	2.31

The actual  $L^*$  values fluctuated over time, and didn't establish a consistent pattern for either treatment. The fluctuation can be attributed to the changing conditions in each individual package, as well as starting formula variability.

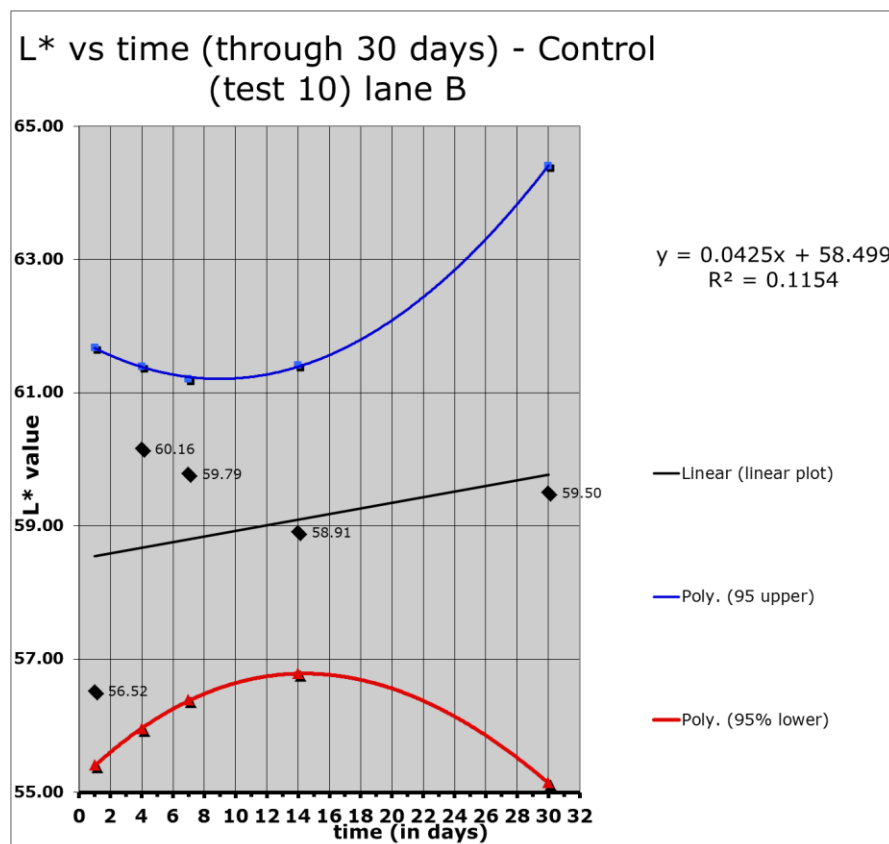
Entering the  $L^*$  values from Table 5.19 above into the kinetics data input sheet (Tables 5.20 – 5.25) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the

product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey color or concentration of pigments (which is also an indication of moisture loss).

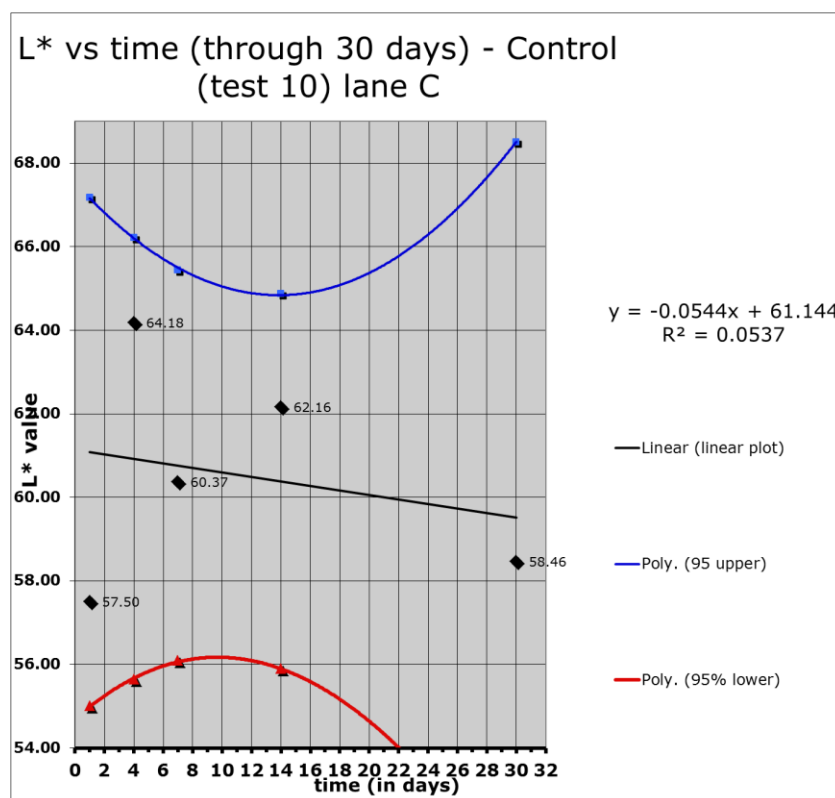


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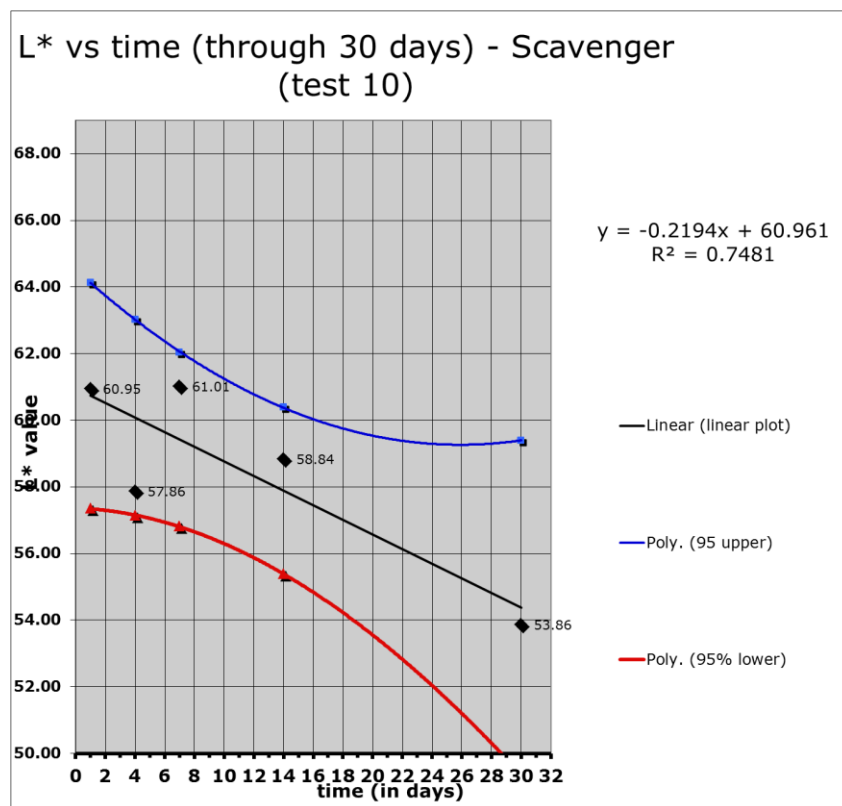
365

[illegible]

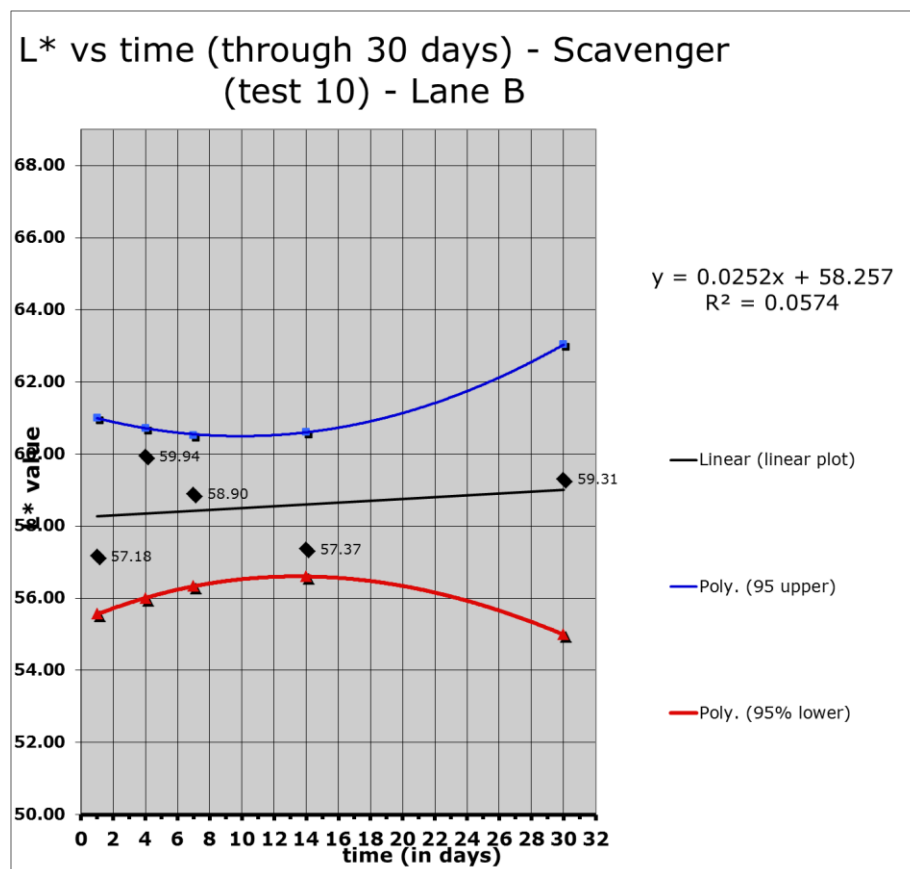
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[illegible]

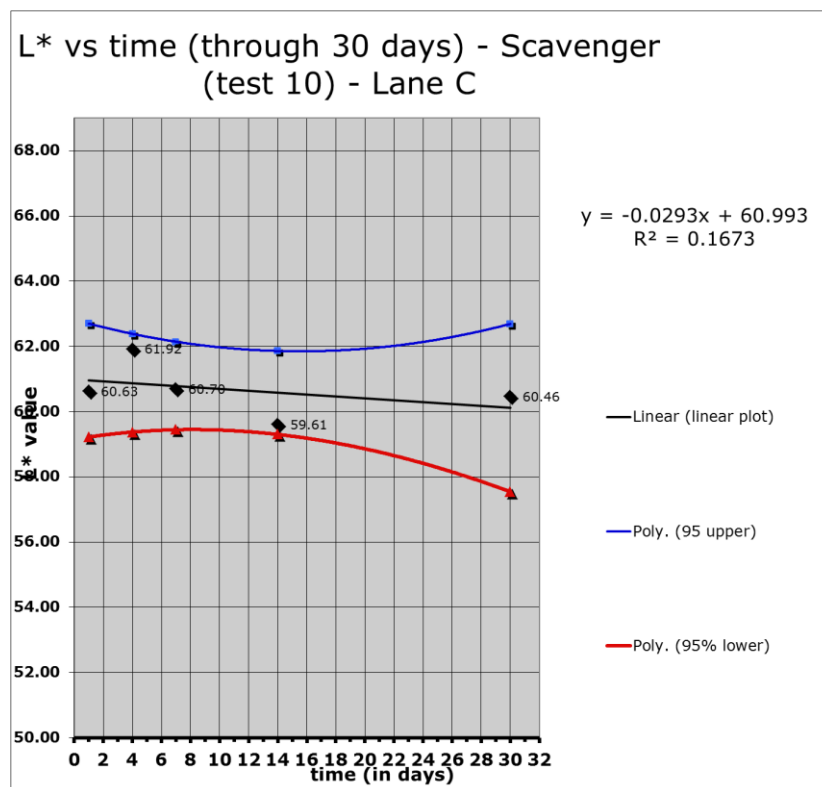
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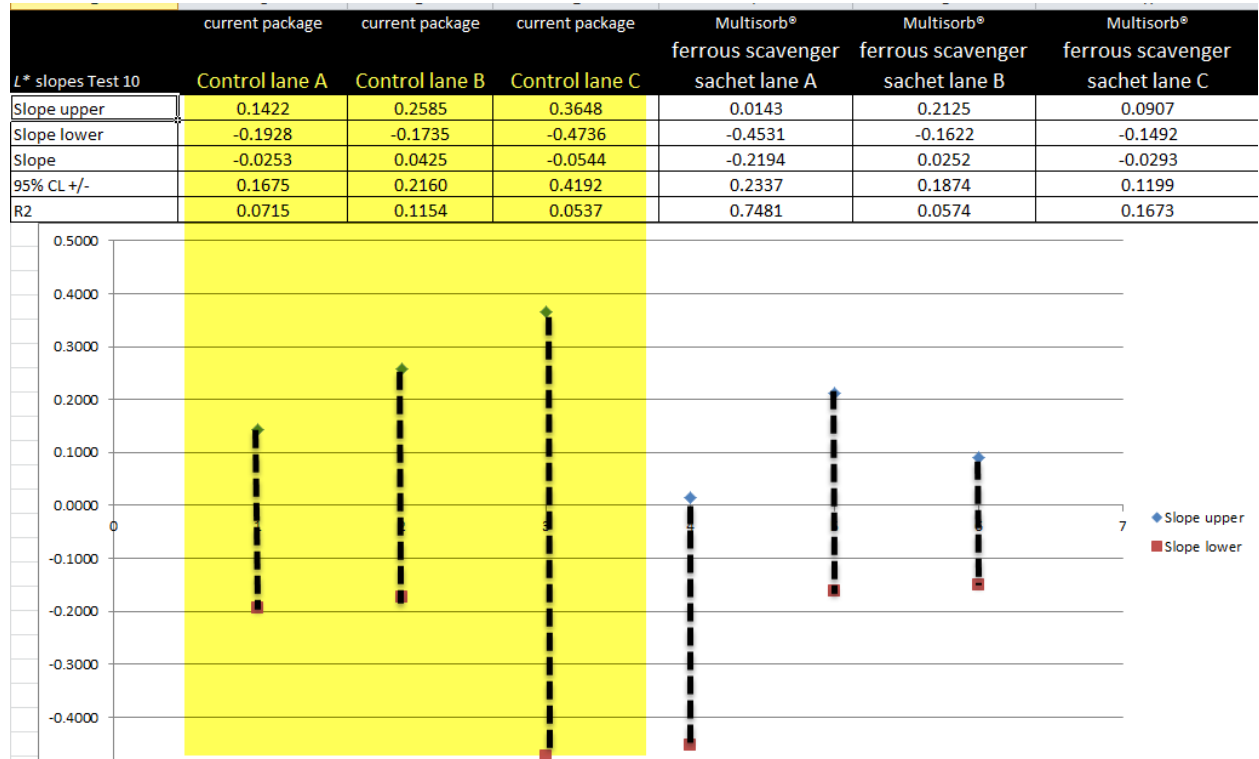
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**Table 5.26**  $L^*$  Slope,  $\pm$  95% CL slope with upper (slope + 95% CL) and lower (slope - 95% CL) for all formulas in test 10 as established by Labuza' Reaction kinetics shelf life model



There is less variability in the scavenger package predicted  $L^*$  slopes over time compared to the control. The control package demonstrates a greater likelihood of positive slopes (increasing lightness or fade) over time; however there is overlap in predict slopes for both treatments in all lanes which make the differences not statistically different.

The  $L^*$  value measurements has some correlation with consumer observations and influenced the pair preference (discussed further in section 5.1.8) In a study of sliced ham with a 10 cc oxygen absorbing sachet, Chaiyapechara, Meng and Hotchkiss (1988) experienced a similar  $L^*$  outcome. When compared to a vacuum packed ham (with no scavenger), the  $O_2$  scavenging sachet package maintained better color retention measured by Hunter L value. Both packaging L values increased over time (indicating a lightening or loss of color), but the scavenger package was consistently 2-3 points lower than the non-scavenger package at each compared day. The reported range of L values for the scavenger package over time were approximately  $L = 60$  to  $64$ , while the non-scavenger

packages were approximately  $L = 61$  to  $67$ . The differences measured in  $L$  value in this study corresponded to visual differences of ham being less pink and lighter grey for the non-scavenger packages. In this sliced ham study, the  $a^*$  values initially dropped after day 1, but improved later in the study for the packages with a scavenger. The higher  $a^*$  values measured later in the study correlated to better color as observed by the naked eye. (Chaiyapechara, Meng and Hotchkiss, 1998)

## **5.1.6 Visual observations Test 10**

### **5.1.6.a Visual observations of the flat format sandwiches in Test 10**

The control package flat ham sandwiches used for  $L^*a^*b^*$  analysis had signs of visual discoloration at all ages of product in the study (Appendix J.1 – J.8). The ham packaged with the oxygen scavenging sachet remained pink throughout the study (Appendix J.1 – J.8). Because of the greater surface area of exposure, the visual differences between the control and scavenger packaged samples were more easily detected compared to the retail wedge format. The potential for greater discoloration exists on the flat format packages compared to the wedge shape because of the increase in product to package ratio (1:1.8 for flats, 1:1 for wedges – section 3.14). As observed by Møller et al., increased headspace is critical to the amount of  $O_2$  available. As headspace area increases, the amount of  $O_2$  increases in the headspace and discoloration develops (Møller et al., 2002). Consistent with previous tests, the sample nearest the light source (referred to as lane A throughout the study) showed greater discoloration throughout the refrigerated shelf life study.

On careful inspection of the flat meat discolored control samples, the discoloration is inconsistent throughout the slice (Appendix J.4-J.8). This is likely attributed to the variation of the muscle and fat composition throughout the slice. It is also observed that the outer edge of the product is more prone to discoloration. This is possibly explained by the exterior of the meat log having greater exposure to light prior to slicing. The outer casing is an oxygen barrier, but allows light through. However given that the length of time the log is exposed to light before being placed in a closed lid combo bin at the ham manufacturer (approximately 1.5 days from the time of stuffing the log, cooking, and



packing) and length of time exposed to light prior to slicing for the sandwich (approximately 2 hours), this is unlikely. There is also the potential of entrained oxygen (trapped during the blending process of the meat and not effectively removed during the vacuum process) in the log which could react with the log exposure to light and affect the color stability of the ham even before it is sliced.

Using the flat meat format improves the chromameter measurements, but also introduces another variable in light exposure and color interpretation. In this case, the bias it creates is the consumer viewed wedge sandwich may be more favorable on color because of decreased surface area for viewing and fewer reference points within the same slice. Discoloration is easier to spot on the flat meat samples because it is not uniform across the slice based on fat and muscle content. In the wedge format, the observation is the exposed area often looks more uniform, so if discoloration is mild and in the form of looking faded but not discolored it might not be as obvious until the area under the label is exposed. Differences in light refraction (the flat meat has a smooth surface versus the wedge cut creates a rougher surface which could affect light reflection) also favors appearance in the wedge format.

#### **5.1.6.b Visual observations of the wedge format ham Test 10 (researchers)**

Visual observations were conducted on the wedge sandwiches (packaging unopened) prior to presenting to consumers, and on the flat meat packages (removed from the package) during the  $L^*a^*b^*$  analysis.

For the consumer pairs, the day four sample of the control visually were duller in appearance with slight grey tones compared to the more vivid pink of the scavenger sample (Appendix J.1). Overall, the control sandwiches did not appear to be discolored at day 4.

At day 7, the difference in appearance was less detectable between the control and scavenger sachet packages. For the day 7 pairs, both samples are pink, but the control

sample appears darker while the scavenger sample appears lighter (Appendix J.2). This visual observation correlates with the consumer preference as the strength of the preference at day 7 was not as strong as day 4, and in favor of the control package (further reviewed in section 5.1.8).

For the day 30 pair, the scavenger sample remains consistently pink, while the control sample is a mix of grey / washed out color combined with some pink. (Appendix J.3)

Consumers were presented with actual sandwiches as they would receive if purchased in a convenience store. Care was taken to match manufacturing and storage practices typical to Deli Express<sup>®</sup> sandwiches in the marketplace. Visual inspection of both the wedge and flat format control packages (MAP only) demonstrated ham discoloration characteristic of a level deemed unacceptable for EA Sween Company.

### 5.1.7 Cooler temperature Test 10

The average cooler temperatures were lower than the desired range of 3.3° to 5° C and were inconsistent compared to each other (Table 5.27).

**Table 5.27** Cooler temperatures over the course of the 30 days study test 10

Cooler	min (C°)	max (C°)	average (C°)
A	-0.5	8.6	2.9
B	-2.5	8.5	1.2
C	-3.5	8.0	0.0
D	-3	7.0	-0.4
E	-2.5	6.0	2.1
F	-4.5	3.0	-0.6

Given these were a part of a test and not the typical retail cooler which is opened consistently during the course of the day, the average cooler temperature was lower than a typical retail cooler at the same settings. Also, not being completely full of product contributed to a lower temperature. While this can be significant as temperature can

increase the rate of the reaction, previous testing (Hunt et al., 2012) would support that lower cooler temperature have not been linked conclusively to improved color scores. As defined by the AMSA, non-abuse temperatures are 0° to 2 C° (Hunt et al., 2012). While the maximum temperatures reached were in the abuse range (Table 5.27), the amount of time spent at this temperature was very short (less than 2 hours) over the course of the 30 day refrigerated shelf life. The lack of high correlations of  $L^*$  and  $a^*$  with time for any treatment, and the temperature fluctuation which is real, in any refrigeration display, adds to the problem of company treatments.

### **5.1.8 Consumer test results Test 10**

#### **5.1.8a Consumer test results – CLT quantitative portion**

The key findings of the consumer study were 1) consumers significantly preferred the ham and cheese sandwich at day 4 with the oxygen scavenger because the control was deemed too light in color; 2) day 7 the preference shifted to the sandwich with control packaging (no scavenger) but the color of the scavenger packaged sample was within the range of being satisfactory, 3) there was no preference at day 30 for either sample (both had equally poor ratings), 4) neat sandwich assembly was a key decision criterion. Sandwiches that appeared assembled sloppily were viewed as very negative and influenced sandwich preference selection, 5) Consumers range of acceptability on pink color was large (light pink was often described as healthier, while darker pink was described as less fatty) A summary of key findings for all age pairs is provided in Figure 5.22. Values not sharing an uppercase letter are significantly different at the 95% confidence level ( $p < 0.05$ ). Values not sharing a lowercase letter are significantly different at the 90% confidence level ( $p < 0.1$ ). Values without letters indicate no significant difference. For each attribute with significant difference, the values are outlined in green to indicate the preferred option and red for the less preferred option.

## Key Measures Summary

- At Day 4 **Prototype** outperformed Current across all key measures, however, at Day 7 **Current** was significantly more liked and significantly preferred over the Prototype.
- At Day 30 there were no significant differences between the two sandwiches.

	Day 4		Day 7		Day 30	
	Current Day 4	Prototype Day 4	Current Day 7	Prototype Day 7	Current Day 30	Prototype Day 30
n=	110		110		110	
Overall Liking of Appearance	5.8 B	6.5 A	6.7 A	6.2 B	5.3	5.4
T2B Purchase Intent (post tasting)	42% B	62% A	58%	56%	25%	30%
Better than Expected (T2B)	24% B	43% A	45%	35%	17%	16%
Worse than Expected (B2B)	24% A	13% B	13%	14%	46%	44%
% of JAR 70% or better	0%	100%	0%	100%	0%	0%
Preference %	35% B	65% A	58% a	42% b	45%	55%



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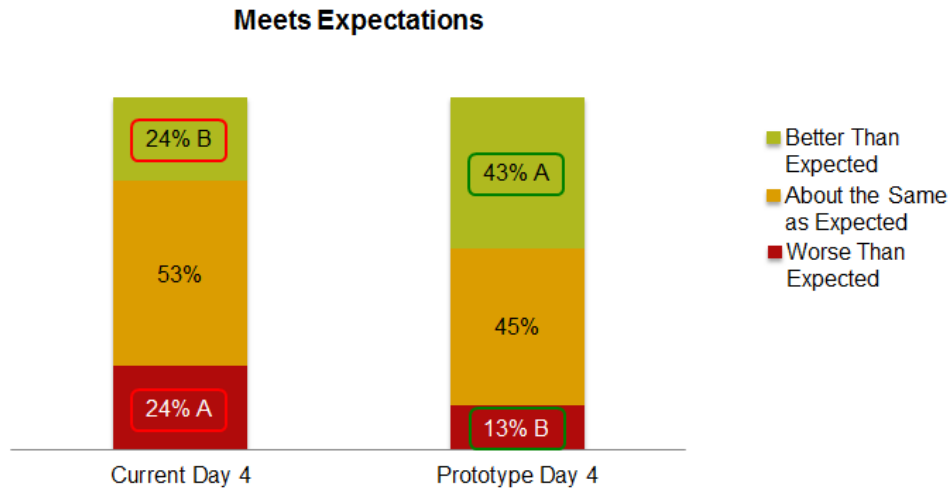
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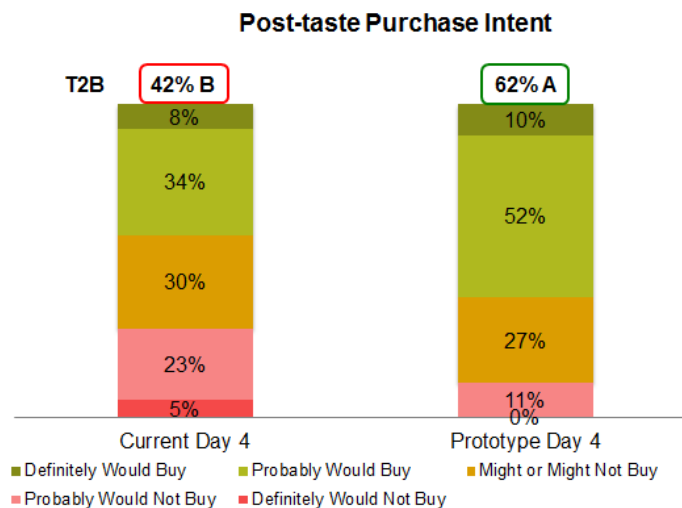
**Figure 5.22** Summary of key measurements from the consumer test

The results for each age pair are further explained as follows.

At Day 4, the prototype (MAP / O<sub>2</sub> scavenger sachet packaged sandwich) outperformed the current (MAP only sandwich) on all key measures. On the question of overall preference (where the consumer had to pick one or the other), the prototype was significantly preferred over the Current control with a 65%/35 % split at the 95% confidence level. The Prototype was significantly more liked than the Current for overall appearance (6.5 vs. 5.8) and meat color (6.8 vs. 5.9). The prototype met expectations significantly better than current based on T2B and B2B analysis (43% vs. 24% T2B and 13% vs. 24% B2B, Figure 5.23).

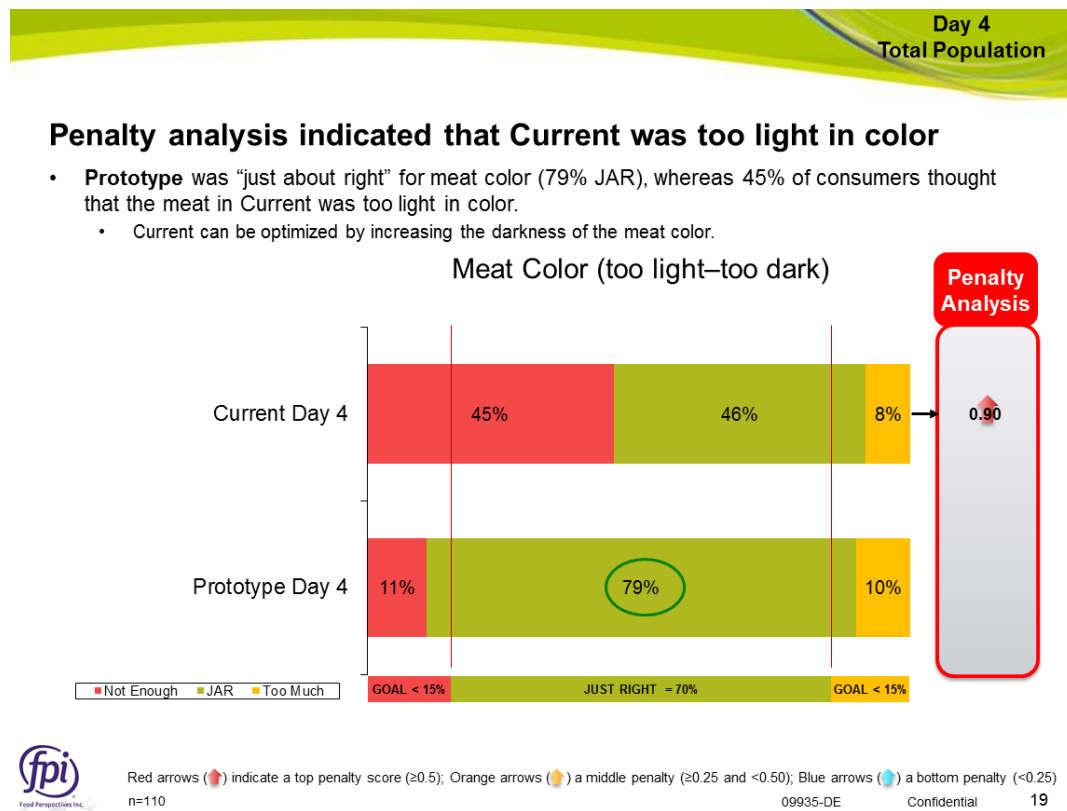


**Figure 5.23** Day 4 results on the question of “Overall, how well does the product meet your expectations of a pre-packaged sandwich”. T2B analysis reveals 43% of the respondents selected “Somewhat Better Than Expected” and “Much Better Than Expected” for the prototype, where only 24% selected the same response to the prototype Purchase Intent was also significantly higher for Prototype compared to Current (62% vs. 42% T2B, Figure 5.24).



**Figure 5.24** Percent of consumer responses to the question of “If this product were available where you shop, how likely would you be to PURCHASE this product?” on day 4

The Prototype was more optimized for meat color than Current with the Prototype receiving JARs response of 79% for meat color, while the control package had 45% of the respondents judge the meat color as too light (Figure 5.25).

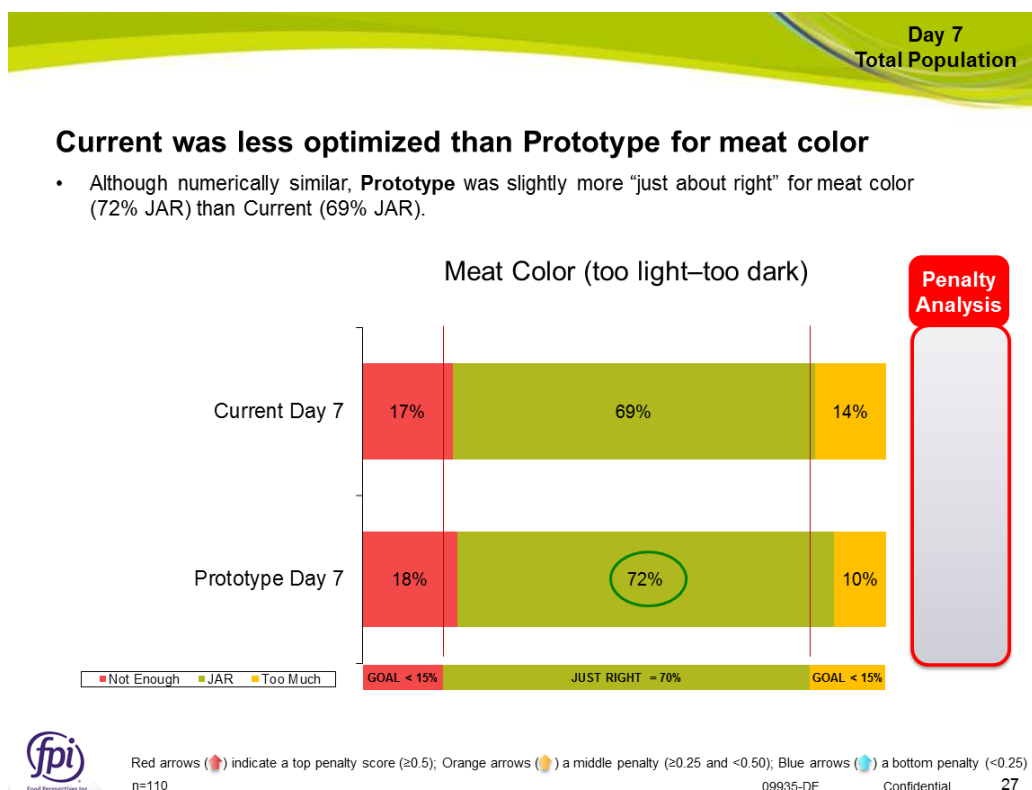


**Figure 5.25** Penalty analysis results for the control (no scavenger sample) compared to the scavenger on the measure of meat color preference at day 4 – Courtesy of FPI®

Actual  $L^*$  values (from the flat package control) confirm that the control was lighter at day 4 ( $\Delta L^* = 1.28$  from the scavenger), with  $L^* = 57.8$  for the scavenger sample and  $L^* = 59.08$  for the control. (Table 5.19 above) This suggests that a small difference in  $L^*$  equates to a consumer perceived visual difference, and suggests that  $L^*$  values at 57.8 are more ideal for color preference on the light and dark scale. The open end responses to the question of “the main reason you preferred this sample” at day 4 were grouped into general categories and included 36 responses for the prototype package of better meat color / quality compared to only 14 for the control, 15 comments of “more meat” for the

prototype vs. 12 for the control, 13 comments of good presentation / assembly for the prototype vs. 10 for the control, and 11 comments of better bread or cheese for the prototype vs. 0 for the control. Though the bread and cheese used was the same for both groups, with minimal variation in the amount of meat used, the placement of ham to give a full “bunched” appearance varies, and influences consumer opinion on all sensory cues they are receiving is assessing the sandwich.

For the day 7 pair, preference switched to the current control package over the scavenger sachet packaged sandwich. While there was no significant difference meeting expectations and purchase intent questions, the preference for the current package sandwich over the prototype at day 7 was a 58/42% split at the 90% confidence level. On the question of “How much do you **LIKE** or **DISLIKE** the **MEAT COLOR** of this product?”, the current package system was more liked for meat color (6.7 vs. 6.3), though the magnitude of the difference was moderate (0.4 hedonics points) and significant at the 90% confidence level. The penalty analysis of the question “Rate the **MEAT COLOR** of this product” revealed that the scavenger package scored slightly higher (72% vs. 69%) for “just about right”, which indicates the strength of the preference at day 7 was much less than at day 4 (Figure 5.26).



**Figure 5.26** Penalty analysis results for the control (no scavenger sample) compared to the scavenger on the measure of meat color preference at day 7– Courtesy of FPI®

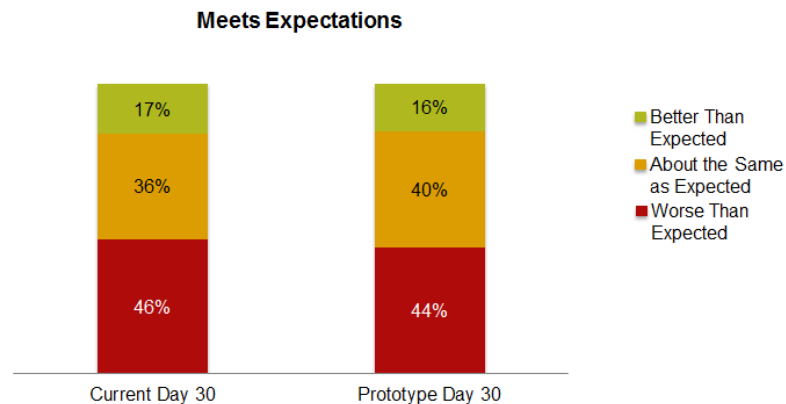
Again, the observed difference in  $L^*$  value for the control and scavenger at day 7 validated consumer comments (the preference between the two was not as strong at day 7 and  $\Delta L^*$  was small between the control and the prototype ( $L^*=0.37$ )). The scavenger at day 7 had the higher  $L^*$  value / was lighter. The open end responses to the question of “the main reason you preferred this sample” at day 7 were grouped into general categories and included 22 comments of better meat color / quality for the current control and 16 comments of the same for the prototype. Additional comments added included 23 responses of more meat for the control vs. 17 for the prototype, 14 comments of Good presentation / assembly for the control vs. 0 for the prototype, and 12 comments of appears fresher for the control vs. 0 for the prototype. The open end responses provide insights into other factors that consumers based their responses on. The overall perception and preference of the sandwich is not just the color of the meat. If the sandwich is poorly made or appears to have a lot of meat, the consumer’s perception of the sandwich is influenced.



At day 30, there was no significant preference for either treatment (55% preferred the prototype and 45% the control). The difference in overall liking of the appearance was similar with both treatments scoring slightly above the neutral response of “neither like nor dislike” (5.4 for the prototype and 5.3 for the control). Using the B2B method revealed that the consumers rated both treatments as “worse than expected” (46% for the control, 44% for the scavenger treatment) to the question of meeting expectation for a pre-packaged sandwich (Figure 5.27).

### At Day 30, neither sandwich met expectations well

- Better than Expected T2B scores were very similar across the two sandwiches (17% vs. 16%).
- B2B scores revealed high degree of dissatisfaction for both sandwiches (46% vs. 44% B2B).



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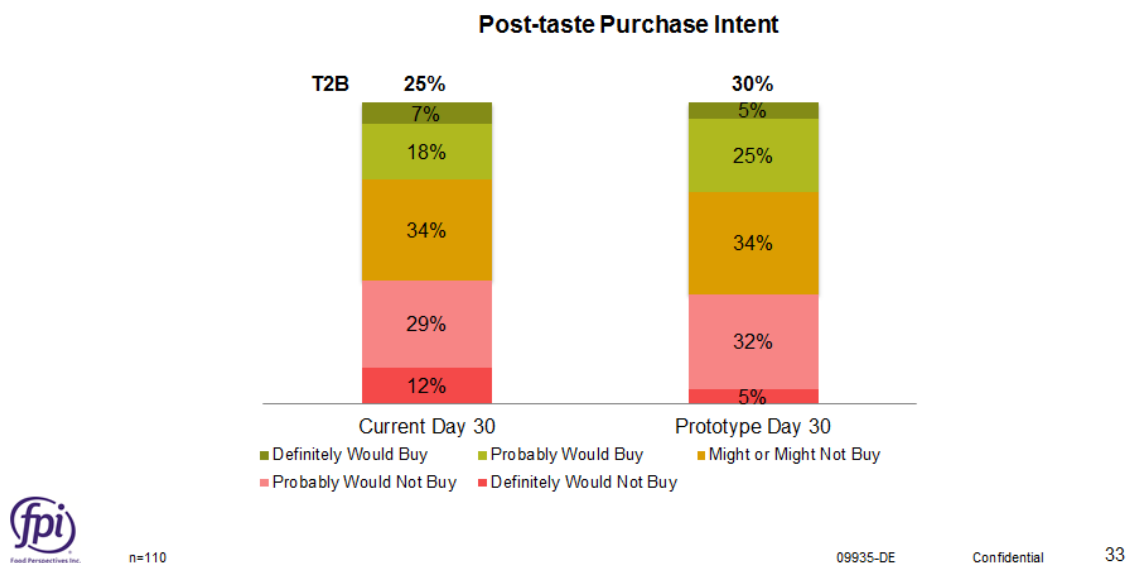
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**Figure 5.27** Day 30 T2B and B2B analysis of the question “Overall, how well does this product meet your **EXPECTATIONS** of a **pre-packaged sandwich?**” Courtesy of FPI®.

Purchase intent for both treatments was low with only a T2B result of only 30% for the prototype and 25% for the control (Figure 5.28)

## At Day 30, Purchase Intent was low for both

- Purchase Intent T2B scores were low for both Current and Prototype (25% vs. 30%).
- B2B PI scores were high for both (41% vs. 37%).

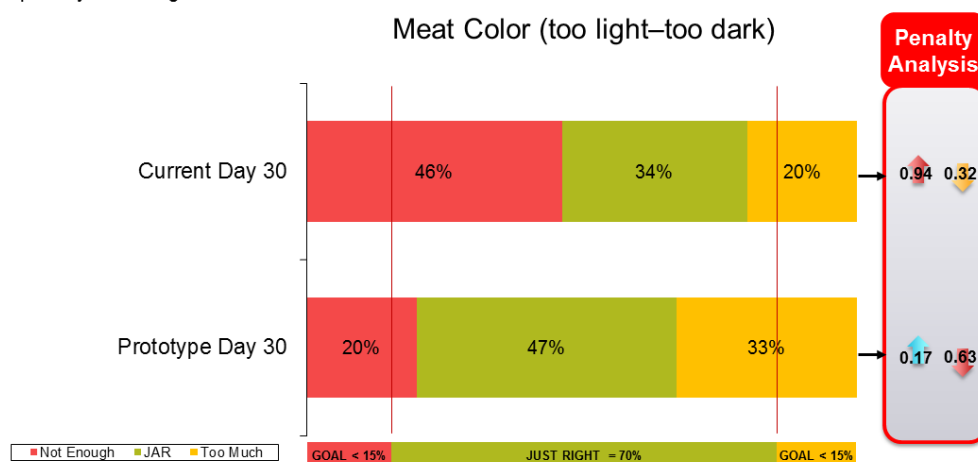


**Figure 5.28** Day 30 T2B and B2B analysis of the question “If this product were available where you shop, how likely would you be to PURCHASE this product?” Courtesy of FPI®.

The prototype was more liked for meat color with a score of 5.5 compared to 5.1 which is significant at the 95% confidence level. Penalty analysis revealed that the color of both hams were less than optimal with 53% (20% + 33%) rating the prototype color as too light or too dark and 66% (46% + 20%) for the control (Figure 5.29).

### Penalty analysis indicated that color was off in both products

- Although penalties for both products were split, indicating consumer segmentation on meat color, Current received a stronger penalty for being “too light”, whereas Prototype received a stronger penalty for being “too dark”.



Red arrows (↑) indicate a top penalty score (≥0.5); Orange arrows (→) a middle penalty (≥0.25 and <0.50); Blue arrows (↓) a bottom penalty (<0.25)

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**Figure 5.29** Penalty analysis results for the control (no scavenger sample) compared to the scavenger on the measure of meat color preference at day 30 – Courtesy of FPI®

Comparing the consumer comments to the  $L^*$  values of both packages at day 30 reveals that the difference in  $L^*$  at day 30 was not very large ( $\Delta L^* = 4.37$ ) with the scavenger being darker ( $L^* = 53.9$ ). The penalty analysis at day 30 for the control was 46% rated as “too light in color”, while only 20% believed the scavenger packed sample was too light. 33% of the consumers thought the ham color was “too dark” on the scavenger sample, with only 20% rating the control as too dark (Figure 5.29). Again the consumer observations are in alignment with differences observed in the packages at day 30.

The consumer observations and comments made at day 4 and 7 of the consumer study did not correlate as well to the measured difference in  $a^*$  value for the paired group. The day 4  $a^*$  value difference between the scavenger and the control was  $\Delta a^* = 7.28$ , where the scavenger had the higher  $a^*$  value and was preferred, compared to a  $\Delta a^* = 5.2$  at day 7

where the control had a lower  $a^*$  value and was preferred (although the strength of the preference was weaker, and only statistically significant at 90% confidence level).

From FPI's perspective, the overall results of the test were inconclusive given that the scavenger packed sandwiches were not selected unanimously for all age pairs. Though many of the control treatments did not have obvious visual discoloration, they were a true representation of retail product in the market. Review of the product with the packaging removed (Appendix J.1-J.8) provides evidence that the discoloration and changes indicative of the photooxidation process are present. The results of this consumer test provided insight as to how the consumer would react to the appearance of the sandwich with the inclusion of an oxygen scavenger to one without.

### 5.1.8b Qualitative peel off results

As it relates to the color of the meat questions, consumer preference varied, with some liking lighter pink color, and others preferring darker color. The preference for either was not strongly in favor of one or the other. Some of the ham was perceived to have a white marbling appearance which was not viewed favorably. Consumer quotes from the peel off session regarding range of acceptable pink color for ham included:

- *"It's a hung jury..." referring to which pink (light or dark) was preferred.*
- *"The ham, to me, looked like too much white marbling on it, and I wondered if it was a bit moldy."*
- *"I prefer a lighter color of [ham]."*
- *"I'm the opposite, I like the dark color because, to me, when meat is turning lighter it's going bad."*

When shown a sandwich with significant discoloration (Figure 5.7 above), the sandwich was seen as unacceptable and a signal that the sandwich was spoiled. Quotes regarding the discolored sandwich were as follows.

- *"Its color is telling me it looks old."*
- *"It looks like mystery meat."*

- *“Looks like it’s spoiled and turned gray.”*
- *“I would not buy that sandwich.”*

### **5.1.8c Consumer observations of the sachet and oxygen scavenging film**

Unaided, most respondents did not comment on the scavenger sachet, even if physically removing it from sticking to the sandwich was required. When prompted about it, the opinions ranged from indifferent to negative. When asked its function, most viewed it as a shelf life aid (most likely dealing with moisture). Any attempt at extending shelf life was often viewed as the product not being fresh. The key observation that came from this study was the sachet initially went unnoticed until pointed out. Given the amount of products in the market today that use scavenger sachets (Pepperoni, Meat Jerky), this was a validation of an overall acceptance, albeit through indifference.

The consumers were also presented with an oxygen scavenger sample with the scavenger built into the film (which makes the overall film appearance gray) (Figure 5.30). The gray tint to the film was immediately noticed and disliked. Most of the comments were around trying to conceal the appearance. Quotes included:

- *“What are you trying to hide?”*
- *“You can’t tell how fresh it is, you can’t tell if the meat is grey or if it’s the tinting and it’s just not appealing.”*



**Figure 5.30** Grey tinted scavenger film (middle) alongside control (left) and scavenger sachet sample (right)

### 5.1.9 Conclusions Test 10

The FPI<sup>®</sup> conclusions to the consumer test were “These results are inconclusive as to the benefit of the scavenger packet from a product aging perspective. No benefit was seen at 4, 7 or 30 days. However, benefits may be seen if tested at several points between 7 and 30 day” (FPI<sup>®</sup> consumer study). The FPI conclusion was based on the fact that the consumers selected the scavenger sachet treatment over the control on day 4 of the shelf life, and the control over the scavenger sachet at day 7. At day 30, the scores for both treatments were indicative of neither treatment being favorable.

The  $a^*$  and  $L^*$  analysis of the predicted slope of the line over time for the scavenger sachet treatment was not statistically different from the control package. Based on this evidence, the scavenger sachet is not a solution for preventing color variation of the ham in a way that will translate to consistent consumer satisfaction at all shelf life days.

However, there is evidence that the O<sub>2</sub> scavenger combined with MAP is creating a more stable, positive  $a^*$  values (higher redness), and lower  $L^*$  values (darkening color) over

time compared to MAP only. There is also evidence that the O<sub>2</sub> scavenger is effectively removing O<sub>2</sub> from the package environment, even with the process of freezing after assembly. This is a positive outcome given the contribution of O<sub>2</sub> to food deterioration over time. Because oxygen in the early stages of refrigerated shelf life is essential for the reaction and the stoichiometry of the reaction is less than 1 to 1 (meaning less oxygen is need to convert nitrosylmyoglobin to metmyoglobin), the ability to minimize or eliminate oxygen is critical.

To the consumer, there are two types of discoloration for ham. The first is a loss of redness replaced with grey and brown colors characteristic of metmyoglobin development. This type of discoloration is viewed as unacceptable and a signal that the product is spoiled and potentially not safe. The second type of discoloration is a lightening or darkening of the pink (or red) color. While not viewed as unacceptable, it does influence preference for the sandwich as revealed in this study. The consumer test results taken in context with the  $L^*a^*b^*$  data provides insight on the link between quantitative values and qualitative observations. In all instances regarding perceived light or dark observations, the  $L^*$  value data supported the consumer observations. For this case when the product was perceived by the consumer to be light, the  $L^*$  score was a higher value while  $L^*$  was low when a dark color was observed. There was not as strong of a correlation between consumer observations and  $a^*$  value. This could be attributed to the fact that most control packages in the test had low oxygen which could have been indicative of variability in the initial manufacture and did not demonstrate significant visual differences. Had the consumers viewed the flat meat packages, which visually were more readily identified as discolored, the outcome may have been different, but irrelevant given that this sandwich appearance is not the go to market strategy. Based on open ended responses in the consumer study, it is also evident that other factors besides color of the meat influence preference responses, with many comments made to the general appearance of the sandwich (neat or messy), and the perceived amount of meat, which was consistent, but varied in appearance based on the degree of “bunching” of the meat. This underscores that the consumer uses many different visual cues in deciding sandwich preference, even when the questions direct comments to the meat color.

Though the results of consumer preference for all age pairs were inconclusive, it was clear that when presented with a sandwich with true discoloration (with characteristic grey or brown tones and washed out appearance), sandwiches were immediately rejected as spoiled. In this study, the only visually grey discolored ham sandwiches occurred in the control packaged sandwiches, with the majority of them in the flat meat format package. It was also clear that the scavenger sachet packaged sandwiches experienced changes in light and dark appearance at different ages (based on actual  $L^*$  values and consumer comments), but the ham color remained consistently pink with absence of grey or brown color development.

The degree of difference in  $a^*$  values ( $\Delta a^* \geq 4$ ) and color observations made in this study (particularly on the flat ham packages in lane A) are consistent with Anderson and Rasmussen's study of sliced ham with Ageless 50 cc oxygen scavenger in which elimination of discoloration was evident through 26 days (Anderson and Rasmussen, 1992).

Judging color with the Chromameter quantitative data provides directional information that can be correlated with consumer comments, but alone can be misleading. While  $a^*$  improved for both treatments at day 30, consumers preferred neither with both products received equally poor ratings on all key measures. It is essential to understand the baseline color score for the product of interest (an  $a^*$  value color score of 12 isn't necessarily bad as a starting value, however evidence would suggest a score of 12 with an initial baseline of 16 would not be favorable), and recommended to pair with visual color observations along with color measurements.

Though the goal of this research was to investigate practical hurdle strategies for preventing photo oxidation in cured lunch meats in prepackaged sandwiches, there are many variables affecting color changes throughout the refrigerated shelf life of the sandwich that also influence consumer perception of the sandwich. Separating out the changes in color attributed to photooxidation from meat formulation variability (muscles used, muscle distribution, fat content), variable oxygen levels in the package due to the MAP process, and changing package conditions (pH, water activity) is very difficult, and makes proving statistical differences challenging. It is important to be aware that photo-oxidation isn't the only reaction driving color variation during shelf life, and if a marbled



product or the appearance of varied muscles is desired, color measurement may be less useful than a sensory panel or consumer study. As the moisture and pH changes over time (as a result of equilibrium between dissimilar components, microorganism growth, and the CO<sub>2</sub> environment in the MAP package), visual changes in the ham and sandwich components occur.

The development of metmyoglobin formation can vary significantly per package as a result of varied oxygen levels per package, the amount of ham exposed (varies per package), and the exact storage position in the retail cooler.

It is well documented that discoloration is an indication to consumers that the product is not fresh or wholesome (Nannerup et al., 2004), and comments from this consumer study support this fact.

FPI also recommended the following based on the results: 1) “Further investigate if the oxygen scavenger provides a benefit at different age points. Be cognizant that the packet does have the potential to reduce consumer appeal and perceptions of freshness”; 2) “Do not incorporate a gray-tinted window on ham and cheese sandwiches because consumers did not find it acceptable and thought that it “hid” the sandwich”; 3) “Focus improvements on creating sandwiches that are assembled more neatly and uniformly” (FPI<sup>®</sup> meat discoloration study 2014). Because the solution needs to apply to all shelf life days, further work is not recommended.

## 5.2 Test 11 - Follow up tests after Test 10

### 5.2.1 Overview of Test 11

The goal of this test was to better understand  $L^*$  and  $a^*$  performance between the control and test package (D-50 O<sub>2</sub> scavenger sachet) at more frequent time intervals during the first two weeks of shelf life. Past tests have resulted in rebounding  $a^*$  scores at day 30, which can add to the variability of predicted slopes over time. This test was set up to evaluate  $L^*$ ,  $a^*$ , O<sub>2</sub> levels and visual observations for 8 out of an 11 day shelf life, where the previous Test 10 evaluated 5 days over a 30 day period.

In Test 10, three shelf life days (4, 7, and 30) were evaluated for consumer preference.

The strongest tristimulus color correlation with preference was with  $L^*$  value. The packaging treatment with the lower or higher  $L^*$  value, of the pair, resulted in consumer preference for that sandwich in each age pair, for example, consumers preferred the scavenger packed ham at day 4 which had a corresponding  $L^*$  value = 57.86 compared to the control at 59.08. The penalty analysis method used in the consumer study at day 30 also revealed that the scavenger packaged ham was too dark in color which corresponded with a low  $L^*$  value ( $L^* = 53.86$ ). The Test 10 results suggest that  $L^*$  scores between 57.8 and 60.6 may be more ideal, and scores above 61 and below 54 are too light or too dark. FPI® recommended in Test 10 further investigations to understand if the oxygen scavenger provides a benefit at different days throughout the shelf life. Applying the Test 10  $L^*$  value insights can provide interpretation to consumer preference.

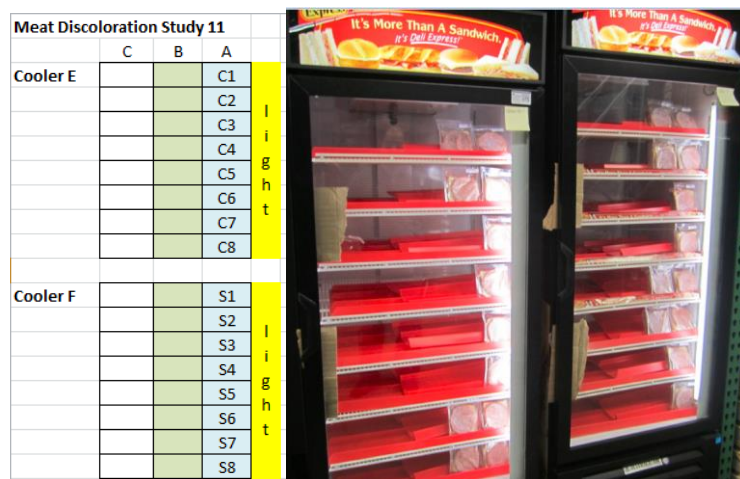
In Test 10, the kinetics models in lane A for both treatments predicts a decreasing  $L^*$  slope over time, as a result of an initial increase followed by a decrease to the starting value at the end of the shelf life (Table 5.20). If the kinetics model predicts a different outcome for both treatments in a shorter time interval (11 days) that is statistically significant, a recommendation of use of a O<sub>2</sub> scavenger with a shorter shelf life test is possible.

Because of the limited number of scavenger packaged samples at day 1 in Test 10, a follow up test was also set up to have a better sample size for the amount of oxygen scavenged at the end of phase 2 (frozen storage) and the start of phase 3 (refrigerated shelf life). The goal is to increase the sample size and test O<sub>2</sub> levels for sandwiches packaged with the D-50 sachet after 7 days of frozen storage (no refrigeration), and 7

days frozen followed by 1 day of refrigeration. In the previous Test 10, the sample size at day 1 of phase 3 (refrigerated shelf life) was n=3 for each treatment, making it difficult to conclude how effective the scavenger was in removing oxygen prior to the start of refrigerated shelf life.

### 5.2.2 Methods and Materials Test 11

Two Beverage Air coolers (Model # LV27 c) with fluorescent lighting were used in this study. Each cooler contained one test variable. For the cooler set, the vertical lane A was loaded one sandwich deep on the front lip (Figure 5.31).



**Figure 5.31** Cooler set up configuration for Test 11. Eight shelves were utilized for a minimum of 8 sandwiches per cooler. Some Lane B slots were loaded to be used in the event of a leaker package.

Sandwiches were removed on each designated day and evaluated eight times throughout an 11 day refrigerated shelf life for oxygen percentage in the package headspace, Ham  $L^*$  and  $a^*$  color analysis (removed from the package) and visual evaluation for lane A control and test variables (photographs documented in Appendix K). A summary of the sample numbers reviewed and corresponding day in shelf life are listed in Table 5.28.

**Table 5.28** Test 11 sample numbers reviewed and corresponding day in shelf life

Calendar				
7/28/2014	Samples produced & placed in frozen storage			
1/30/2015	Samples placed in refrigeration			
	shelf life			
Date	day	Cooler E	Cooler F	
2/1/2015	2	C1	S1	
2/2/2015	3	C2	S2	
2/3/2015	4	C3	S3	
2/4/2015	5	C4	S4	
2/5/2015	6	C5	S5	
2/8/2015	9	C6	S6	
2/9/2015	10	C7	S7	
2/10/2015	11	C8	S8	

The sandwiches used for this study were the flat ham format sandwiches produced for test 10 (on 7/28/14). The materials and packaging used are as is described in test 10.

This product was held for 7 months in frozen storage before the refrigerated shelf life.

The stated frozen shelf life for the sandwiches is 9 months. The temperature of the freezer was approximately -17°C during this time period.

The  $L^*$  and  $a^*$  color analysis (3.10), oxygen analysis (3.12), visual documentation (3.10), and temperature tracking (3.13) is as described in Methods and Materials.

For the phase 1 and 2 testing, the D-50 scavenger was added to production made sandwiches on 7/23/15, and evaluated on 7/30 and 7/31 for oxygen percentages only.

### 5.2.3 Oxygen in the headspace Test 11

#### 5.2.3a Oxygen % in headspace Test 11 from sandwiches made 7/29/2014

After 7 months in frozen storage, followed by 11 days refrigerated storage, the scavenger packages were at 0.0% oxygen, while the control had available residual oxygen at day 4 and 5. (Table 5.29)

**Table 5.29** Residual headspace oxygen percentage in Test 11

control		scavenger	
Day	O2%	Day	O2%
2	0	3	0
3	0	4	0
4	0.193	5	0
5	0.130	6	0
6	0	7	0
9	0	10	0
10	0.001	11	0
11	0	12	0

In the previous Test 10, the control packages had O<sub>2</sub> values between 0 – 0.155%, with 8 of 15 packages having some level of residual oxygen (53%).

### 5.2.3b Oxygen % in headspace Test 11 from sandwiches made 7/23/2015

A summary of the oxygen percentages in phase 1-3 is provided in Table 5.30

**Table 5.30** Oxygen percentages with the D-50 scavenger in phase 1-3

Phase one - immediately following package sealing		Phase 2 - After 7 days of frozen storage		Phase 3 - After 7 days of frozen storage with 24 hours of refrigeration	
CO2	O2	CO2	O2	CO2	O2
20.2	0.082	21.6	0.014	18.5	0.000
20.7	0.053	21.2	0.199	18.7	0.000
20.6	0.001	21.6	0.018	18.5	0.000
20.2	0.501	21.4	0.009	18.8	0.000
20.3	0.091	21.9	0.002	18.8	0.000
20.5	0.004	21.5	0.071	18.8	0.000
19.9	0.106	21.6	0.000	19.1	0.000
20.6	0.021	21.7	0.044	19.0	0.000
20.5	0.061	22.0	0.013	19.0	0.000
20.2	0.025	21.3	0.017	18.8	0.014
20.5	0.058	21.8	0.002	18.5	0.002
20.2	0.026	21.8	0.007	19.2	0.000
20.4	0.060	21.6	0.001	19.4	0.000
20.3	0.047	21.3	0.047	19.2	0.000
<b>average</b>	20.4	<b>average</b>	21.6	<b>average</b>	18.9
<b>min</b>	19.9	<b>min</b>	21.2	<b>min</b>	18.5
<b>max</b>	20.7	<b>max</b>	22.0	<b>max</b>	19.4
<b>range</b>	0.8	<b>range</b>	0.8	<b>range</b>	0.9

Although averages are not as useful given the high package to package variability, in this instance it helps to identify that the average O<sub>2</sub> level after 7 days of frozen storage (0.032%) is roughly half of the initial level at the end of phase 1 (0.081%). Within 24 hours of refrigeration following frozen storage, the O<sub>2</sub> scavenger packaged sandwiches proceed close to zero (Table 5.30).

#### 5.2.4 $a^*$ analysis Test 11

The measured  $a^*$  values for both package treatments is listed in Table 5.31.

**Table 5.31**  $a^*$  value scores for the control and scavenger packages in Test 11 (left). Test 10 results are on the right for reference.

Test 11 $a^*$ results			
Day	Control $a^*$	Scavenger $a^*$	$\Delta a^*$ (scavenger - control)
2	12.33	17.98	5.65
3	8.70	13.80	5.10
4	6.79	16.24	9.44
5	7.47	15.36	7.89
6	9.24	15.64	6.40
9	8.26	14.56	6.30
10	7.41	15.24	7.83
11	10.63	16.99	6.36

Test 10 $a^*$ results lane A			
LANE A day	Control lane A $a^*$	Multisorb lane A $a^*$	$\Delta a^*$ (Multisorb - control)
1	14.01	17.19	3.18
4	10.98	18.26	7.28
7	11.05	16.25	5.20
14	9.32	17.01	7.69
30	14.26	17.80	3.54

The Test 11  $a^*$  values are similar to Test 10 in that they both demonstrating high variability from day to day, with a large  $\Delta a^*$  between treatments for each age pair. However Test 11 had more pairs with a  $\Delta a^* > 4$ .

Entering the Test 11  $a^*$  values from Table 5.31 above into the kinetics data input sheet (Tables 5.32 – 5.33) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984).

1. Raw Data:		
# data pairs Total=	8	This is automatically counted
Y units	a*	Control package lane A
X units	days	

STATISTICS																
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry (y-yi-yes)*^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(y-yi)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
	12.33	2.0	152.03	12.33	9.21	4.00	12.33	9.21	9.76	18.06	24.66	4.00	12.08	6.33	5.75	9.21
	8.70	3.0	75.63	8.70	9.12	9.00	8.70	9.12	0.18	10.56	26.09	9.00	11.58	6.66	4.92	9.12
	6.79	4.0	46.15	6.79	9.04	16.00	6.79	9.04	5.05	5.06	27.17	16.00	11.14	6.94	4.21	9.04
	7.47	5.0	55.80	7.47	8.96	25.00	7.47	8.96	2.21	1.56	37.35	25.00	10.80	7.12	3.68	8.96
	9.24	6.0	85.32	9.24	8.87	36.00	9.24	8.87	0.13	0.06	55.42	36.00	10.59	7.16	3.44	8.87
	8.26	9.0	68.28	8.26	8.63	81.00	8.26	8.63	0.13	7.56	74.37	81.00	10.90	6.35	4.55	8.63
	7.41	10.0	54.91	7.41	8.54	100.00	7.41	8.54	1.28	14.06	74.10	100.00	11.20	5.88	5.32	8.54
	10.63	11.0	113.00	10.63	8.46	121.00	10.63	8.46	4.71	22.56	116.93	121.00	11.56	5.36	6.19	8.46
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(y-yi)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average

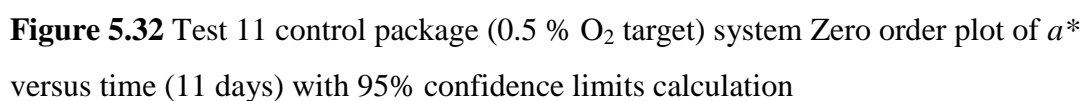
slope=	-0.0829
intercept=	9.3722
rsq=	0.0228
± 95% slope	0.5433
k upper	0.4604
k lower	-0.6262

Equations	
Y =	9.3722 -0.0829 * time

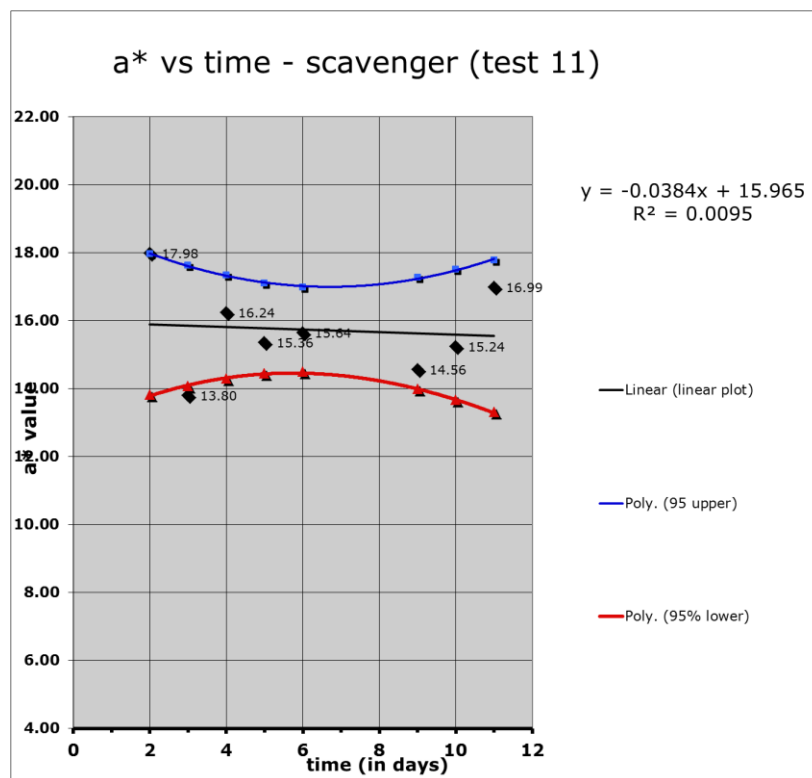
Standard Error	1.98
Sum (yi-yes)	286.97
n	8.00
t 95% 2,n-2=	2.45
x average =	6.25
Sum (xi-xav)	196.69
(Sum x)^2	2500.00
Sum(y^2)	651.11
sum y	70.83
Sum (xi*yi)	436.09
sum x	50.00
sum (X^2)	392.00





**Table 5.33** Test 11  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the scavenger sachet package in Lane A

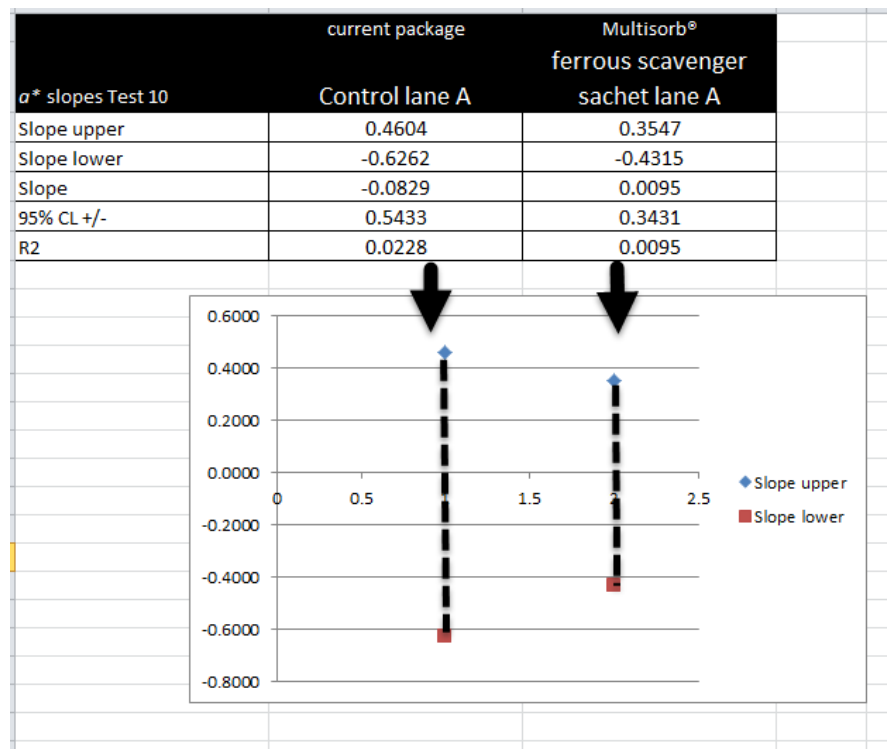
1. Raw Data:															
# data pairs		Total=		8 This is automatically counted											
Y units		a*		Scavenger sachet package lane A											
X units		days													
STATISTICS															
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry row (y1-yes)^2															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
17.98	2.0	323.40	17.98	15.89	4.00	17.98	15.89	4.39	18.06	35.97	4.00	17.97	13.81	4.16	15.89
13.80	3.0	190.44	13.80	15.85	9.00	13.80	15.85	4.20	10.56	41.40	9.00	17.63	14.07	3.56	15.85
16.24	4.0	263.63	16.24	15.81	16.00	16.24	15.81	0.18	5.06	64.95	16.00	17.33	14.29	3.05	15.81
15.36	5.0	235.83	15.36	15.77	25.00	15.36	15.77	0.17	1.56	76.78	25.00	17.11	14.44	2.67	15.77
15.64	6.0	244.51	15.64	15.73	36.00	15.64	15.73	0.01	0.06	93.82	36.00	16.98	14.49	2.49	15.73
14.56	9.0	211.99	14.56	15.62	81.00	14.56	15.62	1.12	7.56	131.04	81.00	17.26	13.97	3.29	15.62
15.24	10.0	232.16	15.24	15.58	100.00	15.24	15.58	0.12	14.06	152.37	100.00	17.51	13.65	3.85	15.58
16.99	11.0	288.55	16.99	15.54	121.00	16.99	15.54	2.09	22.56	186.85	121.00	17.78	13.30	4.48	15.54
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predict average
slope=												-0.0384			
intercept=												15.9646			
rsq=												0.0095			
± 95% slope												0.3931			
k upper												0.3547			
k lower												-0.4315			
Equations															
Y = 15.9646 -0.0384 * time															



**Figure 5.33** Test 11 scavenger sachet package (0.5 % O<sub>2</sub> target) system Zero order plot of  $a^*$  versus time (11 days) with 95% confidence limits calculation

Applying the reaction kinetics model for food quality changes established by Labuza (in this application loss of redness as measured by  $a^*$  over time) establishes a predicted range (upper and lower) for the rate constant ( $k$ ) for both treatments at the 95% confidence level (Section 3.20 Methods and Materials). A summary of the rate constant ( $k$ ) overlap between treatments is provided in Table 5.34. Any overlap in rate constant ( $k$ ) values as predicted by the reaction kinetics shelf life model is not statistically different from each other.

**Table 5.34**  $a^*$  rate constant ( $k$ ) upper and lower for both applications in Test 11 in lane A as established by Labuza' Reaction kinetics shelf life model



Over 11 days, the control predicted  $a^*$  value slopes are not statistically different compared to the scavenger treatment. The scavenger treatment has a narrower range of potential slopes compared to the control (greater predictability), but the range in this test (slope  $\pm 0.3431$  at the 95% confidence level) is larger than the predict range in Test 10 which demonstrated slope variability of  $\pm 0.01197$  (lane A) at the 95% confidence level in the scavenger treatment. The predicted y intercept (time 0) for the scavenger in this study is 15.96 compared to 9.37 for the control, resulting in a  $\Delta a^* = 6.59$  (Figures 5.32-

5.33 above). Though the kinetics model predicts a trend line for each treatment that does not overlap over the 11 day period and has a large  $\Delta a^*$  between treatments that should correlate with visually observed differences ( $\Delta a^* \geq 4$ ), the variability in the actual  $a^*$  values recorded results in a broad range of predicted outcomes that are not statistically different from each other.

### 5.2.5 $L^*$ values Test 11

The variability of  $L^*$  values across all treatments over time is large (Table 5.35).

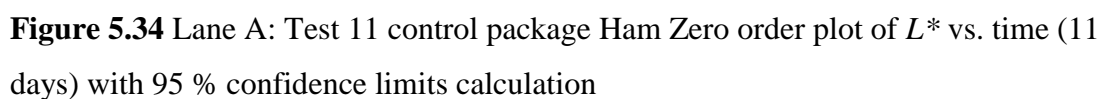
**Table 5.35** actual  $L^*$  color scores for both test and control packages in Test 11. Using insights from Test 10, demonstrates multiple changes in  $L^*$  interpretation

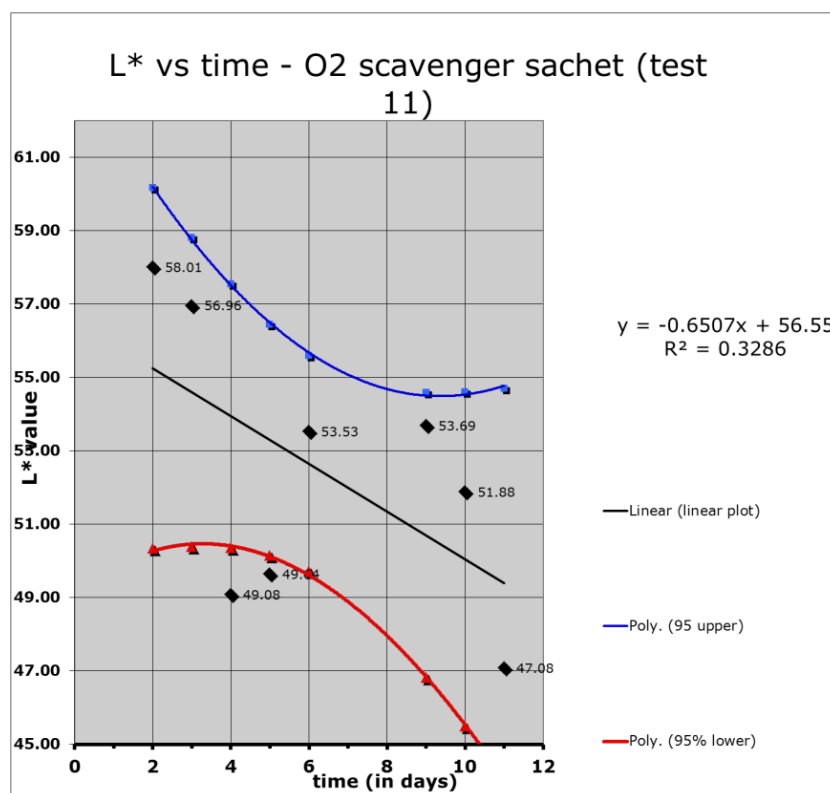
Shelf life day	Control $L^*$	Scavenger $L^*$	$\Delta L^*$ (scavenger - control)	interpretation
2	58.90	58.01	-0.89	control is lighter
3	52.49	56.96	4.48	scavenger is lighter
4	52.91	49.08	-3.83	control is lighter
5	55.61	49.64	-5.97	control is lighter
6	51.52	53.53	2.01	scavenger is lighter
9	52.66	53.69	1.03	scavenger is lighter
10	54.54	51.88	-2.66	control is lighter
11	51.75	47.08	-4.67	control is lighter
12	52.91	51.57	-1.34	control is lighter

With the exception of day 2, the actual recorded  $L^*$  values in this test are much lower than the values from Test 10 which had a minimum and maximum scores between 56.52 – 61.52 across both treatments (Table 5.19 in previous test). Based on the consumer preference learnings from Test 10, the consumer would find both treatments unacceptable at all points beyond day 4 ( $L^* \geq 4$ ). This outcome introduces yet another variable in length of frozen storage to consider for finding viable solutions for preventing unacceptable color changes to the sandwich over time.

Entering the  $L^*$  values from Table 5.35 above into the kinetics data input sheet (Tables 5.36 – 5.37) provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides (Labuza, 1984). For  $L^*$  value, a positive slope indicates a lightening of the

product (interpreted as greater fade over time), a negative slope indicates a darkening of the product over time. For  $L^*$  scores, a desired outcome would be no change over time. Lightening of the product over time is interpreted as fade; however a darkening of the product could be an indication of formation of grey agent or concentration of pigments (which is also an indication of moisture loss).

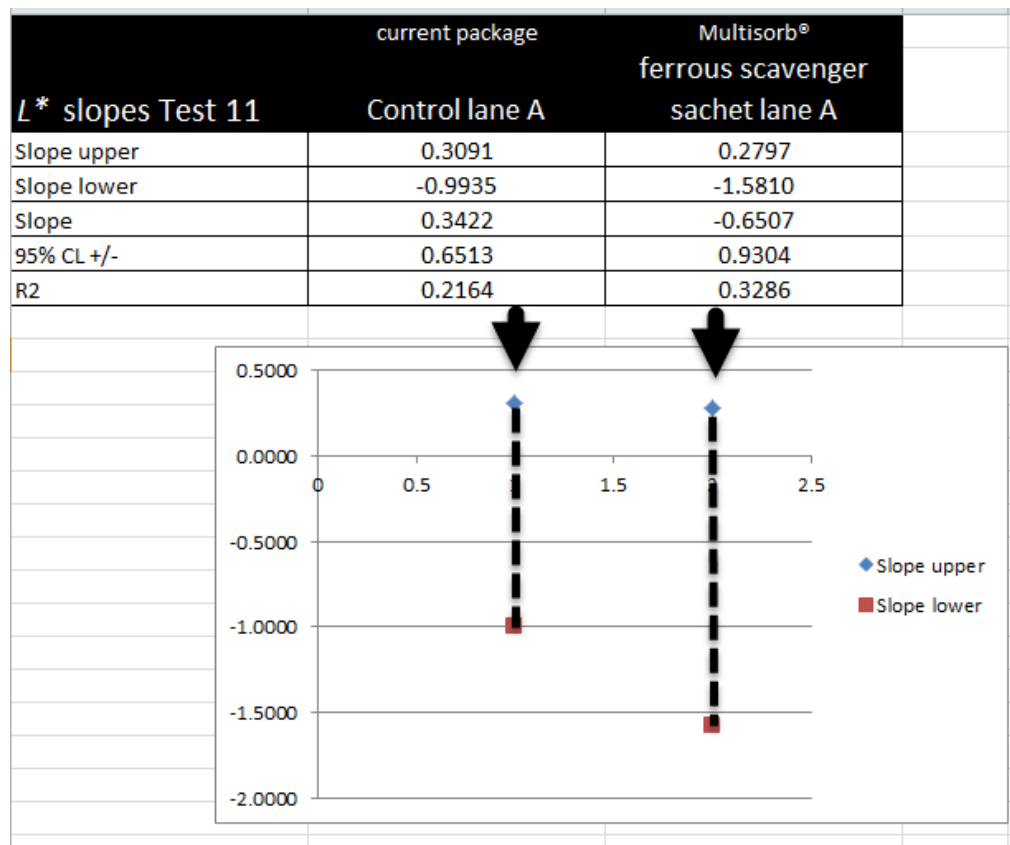
[illegible]

[illegible]

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Applying the reaction kinetics model for food quality changes established by Labuza (in this application changes to contrast (lightening or darkening) as measured by  $L^*$  over time) establishes a predicted range for the slope (upper and lower) for all treatments at the 95% confidence level (Section 3.20 Methods and Materials). Any overlap in predicted slopes (slope  $\pm$  95% Confidence Levels (CL)) between treatments is not statistically different. A summary of the  $L^*$  slope ranges ( $+k$  for lightening over the shelf life,  $-k$  for darkening at  $\pm$  95% CL) between treatments is provided in Table 5.38.

**Table 5.38**  $L^*$  parameter rate constant ( $k$ ) upper and lower for all applications in test 11 all lanes as established by Labuza' Reaction kinetics shelf life model.



Compared to the Test 10 results (Table 5.26 in Test 10), the kinetics model predicts a greater range of outcomes, with the potential for a sharper decrease over time for both treatments which are not statistically different from each other, but suggest a negative impact for both treatments to consumer preference with longer frozen storage.

### 5.2.6 Visual appearance of ham in Test 11

Significant discoloration was observed on all control samples, while the scavenger packaged sandwiches remained visually pink (Appendix K.1 – K.8). Unlike previous testing, the surface discoloration appeared more complete across the surface area compared to controls from Tests 1-10 which often developed discoloration on the outer edges of the meat.

### 5.2.7 Temperatures Test 11

#### 5.2.7a Cooler temperature Test 11

Max temperatures achieved were similar to previous tests, but the minimum temperatures achieved were colder (Table 5.39). With the visual discoloration achieved in the control samples, the lower temperature would provide support that cooler temperature isn't a significant factor with colder temperatures still resulting in discoloration.

**Table 5.39** Cooler temperatures Test 11

Cooler 1	C°	cooler 2	C°
Average	-1.21	average	-2.7
Min	-6	min	-9.5
Max	4	max	5.0

Detailed temperature tracking can be found in Appendix K.2.

#### 5.2.7b Freezer temperature during phase 2

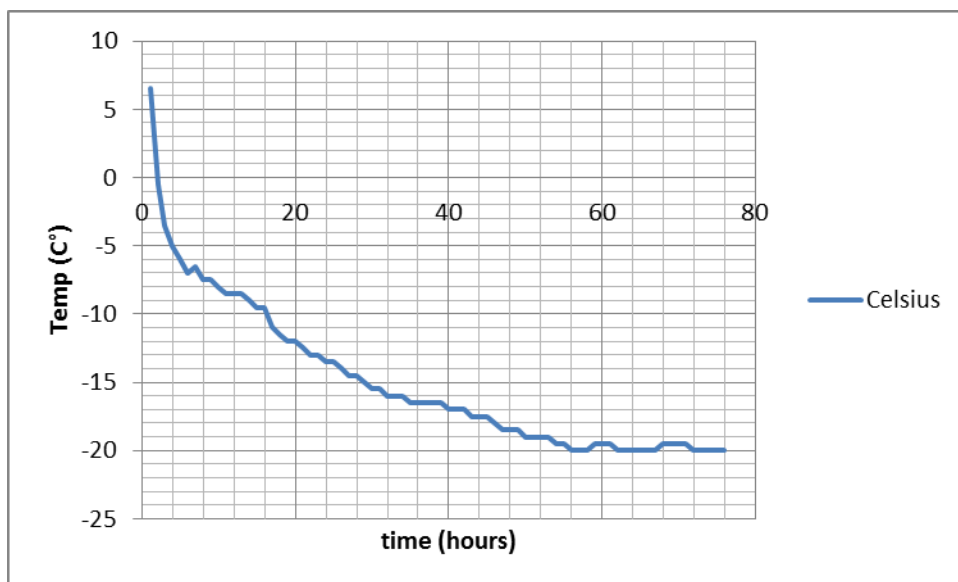
Temperature monitoring during the first 24 hours in the freezer was conducted to understand changes during this window of time. A summary of results is provided in Table 5.40.



**Table 5.40** Temperature inside the sandwich case during the first 24 hours in the freezer

Hour	Celsius
1	6.5
2	-0.5
3	-3.5
4	-5
5	-6
6	-7
7	-6.5
8	-7.5
9	-7.5
10	-8
11	-8.5
12	-8.5
13	-8.5
14	-9
15	-9.5
16	-9.5
17	-11
18	-11.5
19	-12
20	-12
21	-12.5
22	-13
23	-13
24	-13.5

A decrease of 20°C is accomplished in the first 24 hours, with the temperature reaching a study state near -20°C after 50 hours (Figure 5.36).



**Figure 5.36** Temperature changes during the first 24 hours with in the corrugated sandwich case

### 5.2.8 Conclusions Test 11

The day to day variability in a shorter period of time remained large for both treatments on both  $a^*$  and  $L^*$  parameters, and did not result in different predictions in the kinetics model from Test 10. Based on the outcome from this test, another confounder towards identifying a viable solution for a more stable color is identified in the variability of length of frozen shelf life (7 months vs. 3 months from test 10), which resulted in a more visually complete discoloration of the control (Appendix K) and in an  $a^*$  value trend line several points lower than Test 10.

## **6 Conclusions**

Low oxygen gas flushed (MAP) sandwiches containing cured meats can discolor during refrigerated shelf life when exposed to light creating a product that is visually unappealing to consumers and conveys a message that the sandwich has spoiled or is unsafe to consume. The purpose of this research was to explore potential solutions to this problem that can be implemented at the point of sandwich manufacture with the fixed process conditions of MAP followed by frozen storage, frozen distribution, and a 30 day refrigerated shelf life. The product focus was placed on the bestselling cured meat sandwich (Ham and Cheese) for EA Sween Company. My null hypothesis was there are no hurdle technologies available to prevent lunch meat discoloration with the variety of commercially used lighting and refrigeration systems for MAP sandwiches, followed by immediate frozen storage and refrigerated display.

The research objectives were to 1) examine key factors in discoloration of meat and establish the best areas of focus, 2) Measure color changes and oxygen content over time to establish performance differences and statistical significance of potential solutions, 3) Gain insight into consumer preference and opinion of the retail product with potential solutions over current state, 4) Evaluation of the financial impact of potential solutions vs. lost sales, and 5) Make recommendations for EA Sween Company on options for addressing the issue.

### **6.1 Examine key components in the process contributing to unfavorable color changes and establish the best areas of focus**

A better understanding of cooked cured meat pigment and photooxidation was necessary to establishing areas of focus. The pigment of cured meat is nitrosylmyoglobin. When cured meat is cooked, the resulting cooked cured meat pigment is nitrosylhemochrome. The mechanism of nitrosylhemochrome formation is not fully understood, however the basic reaction is when heated, nitrosylmyoglobin is denatured and detached from heme (Varman and Sutherland, 1995). Simultaneously a second mole of nitrite (in the form of Nitric Oxide (NO)) is incorporated into the nitrosylhemochrome molecule complex (Killday et al., 1988). Current evidence indicates that the final molecular form is mono

nitrosylmyochrome with one molecule of NO binding with the color producing heme group and the other molecule of NO with the globin moiety (Hunt et al., 2012 p.9). This compound is sensitive to photooxidation, which is catalyzed by light, resulting in the development of an undesirable brown colored metmyoglobin (Kinsman et al., 1994). Photooxidation occurs when light absorption of heme protein ultimately causes nitrosylmyoglobin to dissociate into nitric oxide and myoglobin. This myoglobin is then susceptible to oxidation to metmyoglobin (Johnston, Knight, and Ledward, 1992). The precise mechanism of photo-oxidation is not known (Johnston, Knight, and Ledward, 1992; Sun et al., 2009), however the rate of oxidation of nitrosylmyoglobin and other heme pigments decreases with lower oxygen partial pressure, and increases with light (Johnston, Knight, and Ledward, 1992).

A significant factor for the initial color of cooked meat products is mainly dictated by the concentration and chemical nature of the starting raw meat material (Ledward, 1992). In the case of cured meats, the amount of nitrite added also affects end color. For the ham in this study, the maximum amount of nitrite allowed by the FDA is found in CFR - Code of Federal Regulations Title 21, Sec. 172.175.

As shelf life proceeds, changes in pH and water content between sandwich components also affect color. Color fading has also been shown to be a partially reversible process suggesting residual nitrite and excess ascorbate play a role after initial color formation (Johnston, Knight, and Ledward, 1992).

Packaging and storage conditions are also significant contributors that affect cured meat discoloration. Research of sliced cured ham lunchmeat has established critical packaging and storage factors to include 1) percent residual oxygen in the package, 2) product to headspace volume ratio in the package (P/H volume ratio), 3) oxygen transmission rates (OTR) of the film, and 4) light intensity (Møller, et al. 2002; Nannerup et al., 2004).

Photooxidation of nitrosylmyoglobin has been found to have a linear dependent relationship in both visible (436 nm) and UV (366 nm) light spectrum (Møller, Bertelsen and Skibsted, 2002). For the ham and cheese sandwich in this research, the P/H volume ratio (1 to 1) and OTR of the films ( $<0.5$  cc/100 in<sup>2</sup>/24 hour) were already optimized. Consumer demand to see the product eliminated the potential of blocking visible light, but given conflicting research on the impact of UV light, the potential of UV blocking

film was considered as a consumer friendly option. Based on the literature review, the areas of focus were established as 1) Ham formulation (inside muscles only, antioxidants), 2) Blocking UV light, 3) Impact of light source, and 4) Further reducing oxygen content in the package.

## **6.2 Color changes and oxygen content over time**

The method selected to measure color changes was a Konica Minolta Chroma Meter CR-410 (Minolta, Osaka, Japan) (3.10). This device was readily available, is portable, and allows for use in the retail setting if required. It is commonly used by many researchers in the evaluation of color changes in cured ham (Anderson and Rasmussen 1992; Cerioli et al., 2009; Chaiyapechara et al., 1998; Møller et al., 2002). The tristimulus values of  $L^*$  and  $a^*$  were used as measures relevant to the visual appearance of the ham. AMSA guidelines were followed regarding aperture size, instrument standardization, and sample thickness and uniformity. The AMSA recommended technique for a MAP product of preparing multiple subsamples from one sample batch was followed, allowing  $O_2$  levels in the package to be obtained and avoiding issues of the packaging film interfering in the color readings (3.10).

The method selected to evaluate statistical differences between treatments was the zero order chemical kinetics data input sheet developed by Dr. Ted Labuza which provides a method that allows a predicted range for the end of shelf life outcomes that is not very different from the range that the point-by-point method provides. Throughout this research, a high degree of variability and scatter occurred with the starting cured ham color (3.11), which the kinetics model helped to provide important insight into identifying general trends. The initial ham color variability can be attributed to fat and muscle content (3.11). This color variability is further compounded by high variability in oxygen content from package to package and by changing conditions in the package over time (pH, water content) that can be expected with a heterogeneous combination of bread, cheese, and meat found in a sandwich. While the chemical kinetics model helped to interpret data that had a high degree of scatter and identify trends, it made proving statistical differences between treatments challenging as the predicted rate constants for color change in most treatments had a broad range of potential outcomes based on the

variabilities in the system. Documentation of visual observations and the consumer preference testing provide additional evidence to consider on the effectiveness of treatments.

The oxygen content of the package is best characterized in three phases following sealing of the package. The first phase is the O<sub>2</sub> level immediately following vacuum, gas flush, and seal. The second phase occurs minutes after sealing through frozen storage which can last from 3 days to 9 months (product is held a minimum of 3 days before distribution to ensure complete freeze). The third and final phase is the start of refrigerated shelf life until consumption (1 to 30 days after refrigeration). While most tests in this study focused on phase 3 O<sub>2</sub> and color ( $L^*$ ,  $a^*$ ) results, understanding phase 1 and 2 performance offers the insight that MAP alone is inconsistent in removing oxygen (3.15).

In Test 3, use of antioxidants and an alternative muscle formulation were examined and found to not result in statistical  $a^*$  and  $L^*$  color differences over time compared to the control ham formulation. While the line of best fit in the kinetics model predicts a loss of redness (decreasing slope) for the control ham (full scale production batch) and inside muscles only ham, and an increase in redness (increasing slope) for the pilot plant control, rosemary added, and fruit extract added hams, overlapping  $a^*$  value slopes were predicted as a result of a high degree of scatter. The kinetic model identifies that if batch differences could be minimized by better supplier control, addition of rosemary or fruit extract to the formula results in a very similar range of predicted outcomes when compared to the current formula (Table 4.57).  $L^*$  values demonstrated an even broader range of overlapping predicted slope outcomes compared to  $a^*$  values, with all applications having a greater likelihood of decreasing over time, indicating a darkening of the ham, although the overall changes were small (Visual discoloration of all proposed ham formulations during the shelf life (Appendix C.1 – C13) supports the conclusions of the statistical analysis that these formulation changes do not result in a meaningful improvement of the ham color. Because the mechanism and molecular nature of the free radical in the photo oxidation of cured meat isn't known, establishing an effective antioxidant and quantity needed is challenging. The current ham formulation is driven partly by consumer demand, but also influenced by economic considerations. The use of

inside muscle (semimembranosus that contains a small portion of darker red pigment referred to as red eye which is found near the femur) and the gracilis muscle in the formulation results in darker red color spots within the ham slice, but is an economical formulation that meets consumer's expectations of good quality. The consumer demand for natural muscle and minimally processed appearance (it is not an emulsion) limits formulation changes that can be made to make improve color consistently across the whole surface. While the potential exists to remove gracilis muscle and red eye from the formula, the added financial impact to the manufacturing cost does not make this a viable option.

The cooler type (open or closed) and light source (LED or fluorescent) are not practical variables for the sandwich manufacturer to control, as it is the retailers choice, however understanding the impact to development of ham discoloration was important from the standpoint of recommended best practices that can be provided to the retailer. In Test 1, the ham color performance differences between the refrigerated storage conditions of an open cooler, closed door with no light, and closed door coolers with LED lights and fluorescent lights, resulted in no statistical performance differences in  $a^*$  and  $L^*$  color parameters. Each cooler had a high degree of variability on both tristimulus color parameters, but the kinetics model revealed general trends that suggest moisture loss in open coolers may result in dehydration of the ham and concentration of the pigments over time (Figure 4.8) and that even in dark refrigerated storage, color variation develops over time (Figure 4.12) which is likely attributed to the ham starting color variation and is compounded by storage variation described above. Visual discoloration was also observed in all coolers other than the darkened cooler (Appendix A.1-A.10), providing additional support to the statistical model that there are not consistent performance differences when using different light sources or open or closed door coolers.

Several UV blocking films were evaluated (3.5, Tests 5, 7) and established that their effectiveness were not statistically different from the control package on the parameters of  $a^*$  and  $L^*$  values over time. While the UV films predicted a lesser potential for negative  $a^*$  slopes (loss of redness) compared to the control package, they demonstrated similar overlapping potential for positive slopes over time making them not statistically different from the control. The  $L^*$  values predicted a similar large range of potential

slope outcomes for both control and UV films, with a greater potential of negative slopes (darkening) in all treatments over time (Table 4.96). Consistent with other studies, visible light proved to be as equally destructive as ultraviolet light in the photo-oxidation mechanism (Møller, Bertelsen, Skibsted, 2002). This result also supports the light source findings. While UV light is absent in LED lighting, the effects of the visible spectrum are still present. A reason that fresh meats may benefit more from LED lighting and less UV exposure is the meat pigment of fresh meat (oxymyoglobin) is not as stable as the cured meat pigment nitrosylhemochrome. Many of the fresh meat studies that found improvement under LED and low UV lighting conditions also contained beef, which has a higher myoglobin content than pork, which may also explain why benefits were seen. Oxygen content over time with MAP only has proved to be highly variable from package to package and difficult to control consistently through the MAP process alone. In Test 2, examination of the design of the evacuation chamber for the Multivac equipment revealed the potential for products in the middle cavities to receive less vacuum (3.19). This package to package variability is compounded by bread slices with a range of thicknesses, resulting in a varied potential for trapped air in the bread. With white bread having a porosity of 64.4 – 84 % with 99% of the pores connected (Wang, 2014); each sandwich package is more unique in O<sub>2</sub> composition in the headspace due to trapped O<sub>2</sub> in the bread. Oxygen scavengers offered a unique potential solution for eliminating oxygen in the headspace, after vacuum processing and sealing, despite the O<sub>2</sub> variability and were a primary focus in this study (Tests 4, 5, 7-11). Both ferrous based and non-ferrous based oxygen scavengers were considered, as well as different absorbing capacities. The non-ferrous based oxygen scavenging film was not effective because of the scavenging mechanism (a free radical generated from UV light exposure) was terminated at freezing temperatures (Test 8). This outcome was validated by overlapping predicted  $a^*$  and  $L^*$  value slope performance over time for treatment vs. control and visual discoloration of the ham between treatments (Appendix H.1-H.6).

Ferrous based oxygen scavenging solutions were evaluated in both the form of an individual sachet (3.2) and incorporated into the film (3.6) (Tests 9 and 10). The D-50 cc sachet in Test 9 demonstrated very little predicted slope overlap compared to the control package. The sachet packaged ham predicted positive slopes (increasing redness) as



compared to the control that demonstrated only negative slopes over time (Table 4.157). However, the package to package variability resulted in a small portion of predicted overlap between treatments making this not statistically different. A key difference from other tests was the observed absence of visual discoloration in the scavenger sachet packaged product compared to the control (Appendix I.1 – I.5). The ferrous based scavenger film demonstrated a similar potential to the sachet for positive slopes, but also demonstrated negative slopes during storage (Table 4.157). The visual appearance of both the scavenger sachet and film packaged ham supported the absence of visual discoloration, making both options of interest for consumer input on the visual color of the meat and packaging appearance.

The ability for any O<sub>2</sub> scavenger to be successful is dependent on quick removal of oxygen in dark storage before exposure to light. While the D-30 cc sachet did not consistently demonstrate complete removal of oxygen throughout the refrigerated shelf life (Tables 4.71, 4.84), the D-50cc sachet was successful in consistently reducing O<sub>2</sub> levels (Tables 4.118, 4.149, 5.8, and 5.29).

While the potential exists for diminished O<sub>2</sub> scavenging capability due to the interference of carbon dioxide with the iron or poor air circulation around the sachet, the process of freezing creates the greatest challenge for the effectiveness of this solution. Freezing the sandwich after packaging resulted in removal of only approximately half of the oxygen present during phase 2 of frozen storage. The O<sub>2</sub> level at the start of phase 3 proceeded to zero in most but not all packages within 24 hours of refrigerating (Table 5.30).

Because of the presence of some oxygen at the start of refrigerated shelf life and light exposure, the potential of discoloration of ham as a result of photooxidation exists in the scavenger sachet solution. A minimum relative humidity of 40% is required for the ferrous based scavenging reaction to work. Salts are added to improve activity at lower temperatures but once -18° C is achieved, measurable O<sub>2</sub> scavenging is not achieved.

Within four hours in the freezer, the temperature creates conditions that are not favorable to the reaction (Figure 5.36).

### **6.3 Insights into consumer opinion of ham color with potential solutions**

The consumer testing revealed inconsistent preference between the D-50 ferrous based O<sub>2</sub> scavenger and control MAP only treatment over time. While the scavenger packaged ham was statistically preferred over the control with a 65%/35% split at the 95% confidence level at day 4 with a Just About Right (JAR) score on meat color of 79% vs. 46% (Figure 5.25); the opposite was true at day 7 where the control was statistically preferred by 58%/42% split at the 90% confidence level over the scavenger. While the JAR responses on ham color at day 7 slightly favored the scavenger 72% vs. 69% for the control, the preference was for the control, with comments made that would suggest other visual cues of the sandwich (like how neatly the sandwich appeared to be assembled) influenced the preference choice. This insight highlights the fact that many visual cues including discoloration impact the consumer's choices, and discoloration could be overlooked if not severe and combined with other visual defects. The consumer test also identified two types of visual interpretation of discoloration of ham. The first is a loss of redness replaced with grey and brown colors characteristic of metmyoglobin development. This type of discoloration is viewed as unacceptable and a signal that the product is spoiled and potentially not safe. The second type of discoloration is a lightening or darkening of the pink (or red) color. While not viewed as unacceptable, it influences sandwich preference (5.1.8c).

Regarding the physical presence of the sachet, most consumers (after the sandwich was opened and the sachet was made visible) unaided did not notice or comment on it, however when it was pointed out, the sachet was not viewed as a signal of the product being fresh (5.1.8d). Given the trends towards fresher and minimally processed foods, further understanding of the consumer response to the sachet is recommended.

The appearance of the ferrous based film solution which had a distinct grey tint was immediately noticed and viewed as an attempt at hiding the appearance of the sandwich. The consumer preference results, combined with the lack of statistical difference in the color analysis over time on the tristimulus color parameters of  $a^*$  and  $L^*$  between the current packaging and the D-50 oxygen scavenger sachet result in accepting the null hypothesis that there are no hurdle technologies available to slowdown or prevent lunch

meat discoloration with the variety of commercially used lighting and refrigeration systems for MAP sandwiches.

## 6.4 Evaluation of the financial impact of potential solutions vs. lost sales

Although acceptance of the null hypothesis makes the financial impact of adding an O<sub>2</sub> scavenger sachet a moot point, there are future research recommendations that keep ferrous based O<sub>2</sub> scavengers of interest. With a capital investment of \$65,000 (for automated sachet placement), and the added cost of approximately \$.03 per sachet / sandwich, the added cost to ham and cheese sandwich varieties (assuming 5 million units) in the first year is \$215,000. This cost is potentially offset by reducing or eliminating shrink (sandwiches that are bought back after shelf life expiration) which has a potential annual savings of \$34,000, which doesn't financially justify the addition. However, the impact to brand image and potential lost sales is not fully understood, and requires further exploration. An intriguing area of potential savings with an oxygen scavenger is to reduce the vacuum time during MAP packaging and increase the speed of the packaging equipment resulting in greater production output per unit of time. While this would lead to an increase in the residual oxygen levels in the package, the scavenger has the potential to offset the oxygen increase. By targeting a higher residual oxygen level (1.0%), there is the potential to generate \$43,000 in annual savings over 5 million units. The current approximate vacuum time required to reduce the headspace to 0.5% O<sub>2</sub> is approximately 2 seconds. Changing to a 1.0% targeted residual oxygen level could save 0.5 seconds per machine cycle, generating 12.6 minutes of additional production time per 112 minute run (Table 6.1). With eliminating shrink and increasing line throughput, a first year savings of \$77,000 would offset the initial year 1 estimated \$215,000 added cost of the scavenger. The savings increases with higher residual oxygen levels.

**Table 6.1** Multivac vacuum time savings

dwelt time to 0.5% O <sub>2</sub> (seconds)	dwelt time to 1.0% O <sub>2</sub> (seconds)	difference (seconds)	number up per cycle	cycles per min	seconds gained per min	min per run	seconds gained per 112	min saved
2	1.5	0.5	8	13.5	6.75	112	756	12.6

## **6.5 Make recommendations for EA Sween Company on options for addressing the issue**

While oxygen scavengers have potential, the testing done in this thesis and the consumer input, combined with the economics does not justify E.A. Sween Company making any changes to the current state package. There is recommended future research in this area.

## 7 Future research

The D-50 cc scavenger sachet was more effective at scavenging  $O_2$  compared to the D-30 cc sachet in a frozen storage system. However, the D-50 cc sachet did not remove all of the  $O_2$  when placed immediately in a frozen storage and as a result, contained some residual  $O_2$  at the start of refrigerated shelf life. Exploring a stronger capacity sachet (more iron) has the potential to improve the amount of oxygen in the frozen condition, but at a higher cost. If oxygen can be eliminated completely prior to any light exposure, the color quality may be more stable over time in the scavenger packaged system, which suggests a possible refrigerated (4 to 5°C) holding prior to freezing. Further exploration of refrigerated dark storage prior to freezing with and without a scavenger, is a suggested area of research. Unfortunately at the current time, the amount of refrigerated space needed to support refrigerated storage for any length of time is not practical. However, other research has demonstrated that cured ham stored refrigerated for 4 days in MAP packaging in the dark was enough to deplete the oxygen needed for photo-oxidation to occur (Anderson et al. 1988) and that with an oxygen scavenger with MAP, only 10 hours of dark refrigerated storage is required (Anderson and Rasmussen, 1992). With the greater potential of trapped air in a sandwich system compared to sliced meat only, an  $O_2$  scavenger in a MAP package has good potential. In this study, it was demonstrated that the D-50 scavenger was capable of reducing  $O_2$  levels to near zero within 24 hours of refrigerated storage (Table 5.30)

Because the visual appearance of ham packaged with the D-50 scavenger demonstrated cured color characterization that appeared more favorable to the researchers over the control package (Appendix 7, 9, 10, and 11), use of a trained descriptive color panel could be an alternative to a consumer study for interpretation of the results.

It would also be a benefit to future research if guidelines could establish levels of metmyoglobin formation leading to consumer rejection. Comments from the consumer study would suggest some consumers prefer lighter ham appearance which introduces the potential that some level of fade as the result of photooxidation may actually improve consumer preference for product. If correlations could be made, the use of spectrophotometry to measure the level of the various pigment staged would be useful.

As demonstrated in Test 11, the length of frozen storage may have an overall impact on color quality in both the control and scavenger packaged sandwiches. Using the same sandwich production lot in both Test 10 and 11 resulted in a decreased  $a^*$  ( $\Delta a^* = 1.99$ ) in the control and a decreased  $a^*$  ( $\Delta a^* = 1.19$ ) with the scavenger. Though this could be attributed to batch variability, the trend lines in Test 11 consistently predicted  $a^*$  values 2 points lower than Test 10 throughout the shelf life, with a difference of 6 months frozen storage between the studies. Based on this, further research into the effect of longer frozen storage is recommended for any potential solutions.

An opportunity also exists for improvements to the MAP packaging equipment design. If greater control could be obtained to ensure similar final  $O_2$  levels for all packages within the evacuation cycle, there is a potential improvement to the consistency of oxygen removed.

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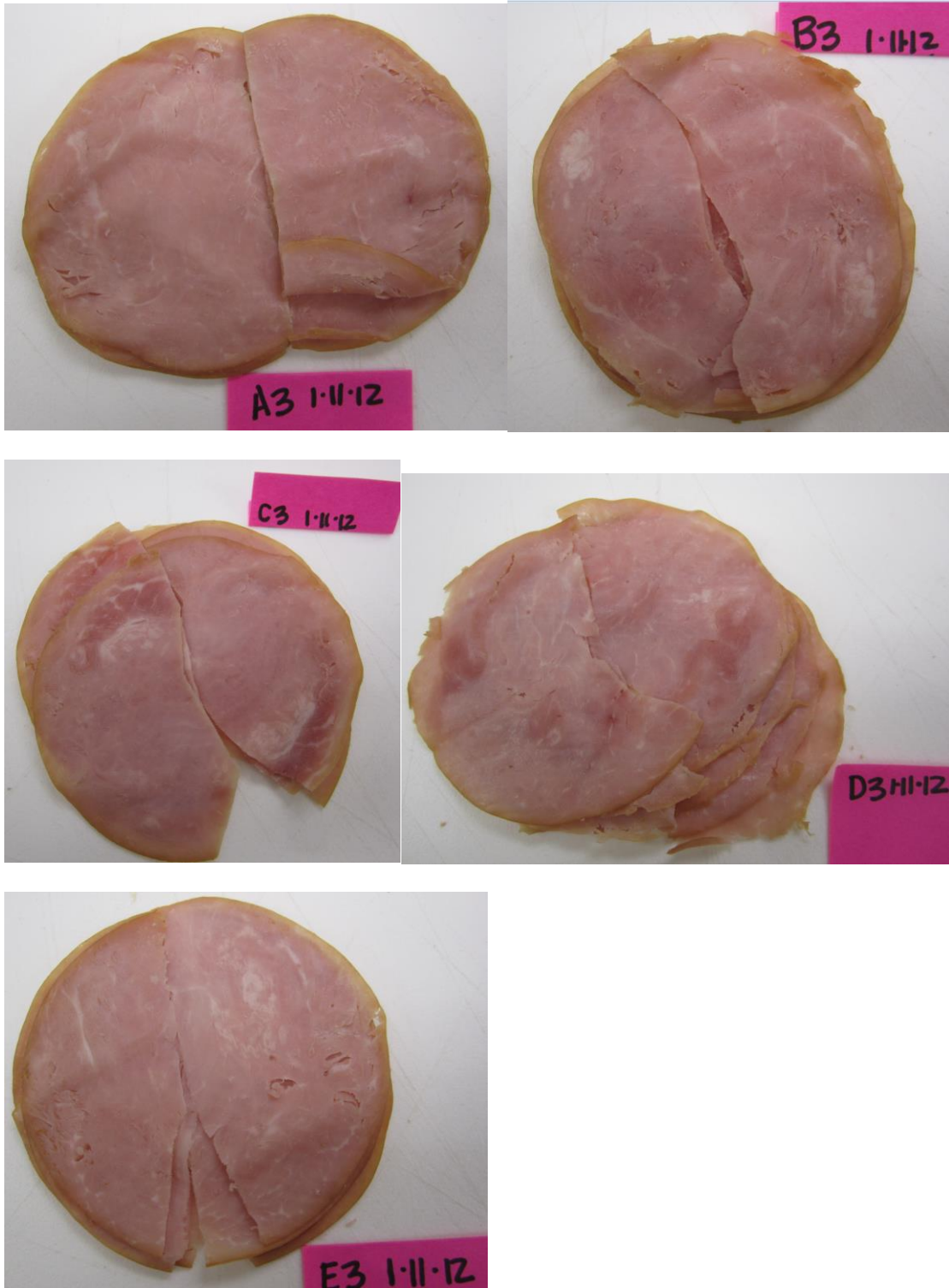
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Yen, J. R., Brown, R. B., Dick, R. L., & Acton, J. C. (1988). Oxygen Transmission Rate of Packaging Films and Light Exposure Effects on the Color Stability of Vacuum-Packaged Dry Salami. *Journal of food science*, 53(4), 1043-1046.

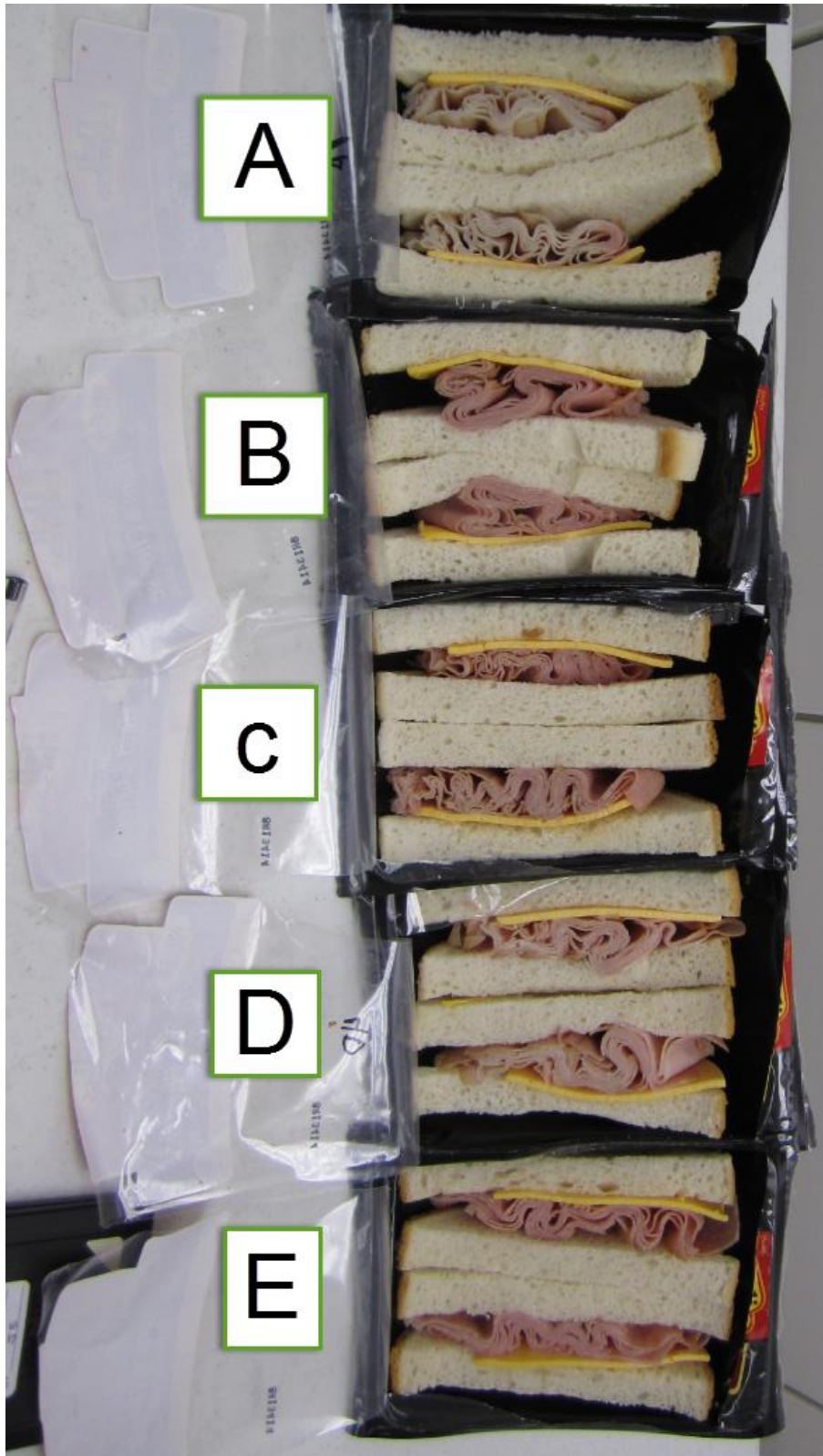
Young, O. A., Priolo, A., Simmons, N. J., & West, J. (1999). Effects of rigor attainment temperature on meat blooming and colour on display. *Meat Science*, 52(1), 47-56.

## Appendix A - Test 1 Cooler and Light bulbs

A.1: Day 5, appearance of the sandwiches in all coolers, of Test 1



**A.2:** Day 6, appearance of the sandwiches in all coolers, of Test 1





A.3: Day 11, appearance of the sandwiches in all coolers, of Test 1

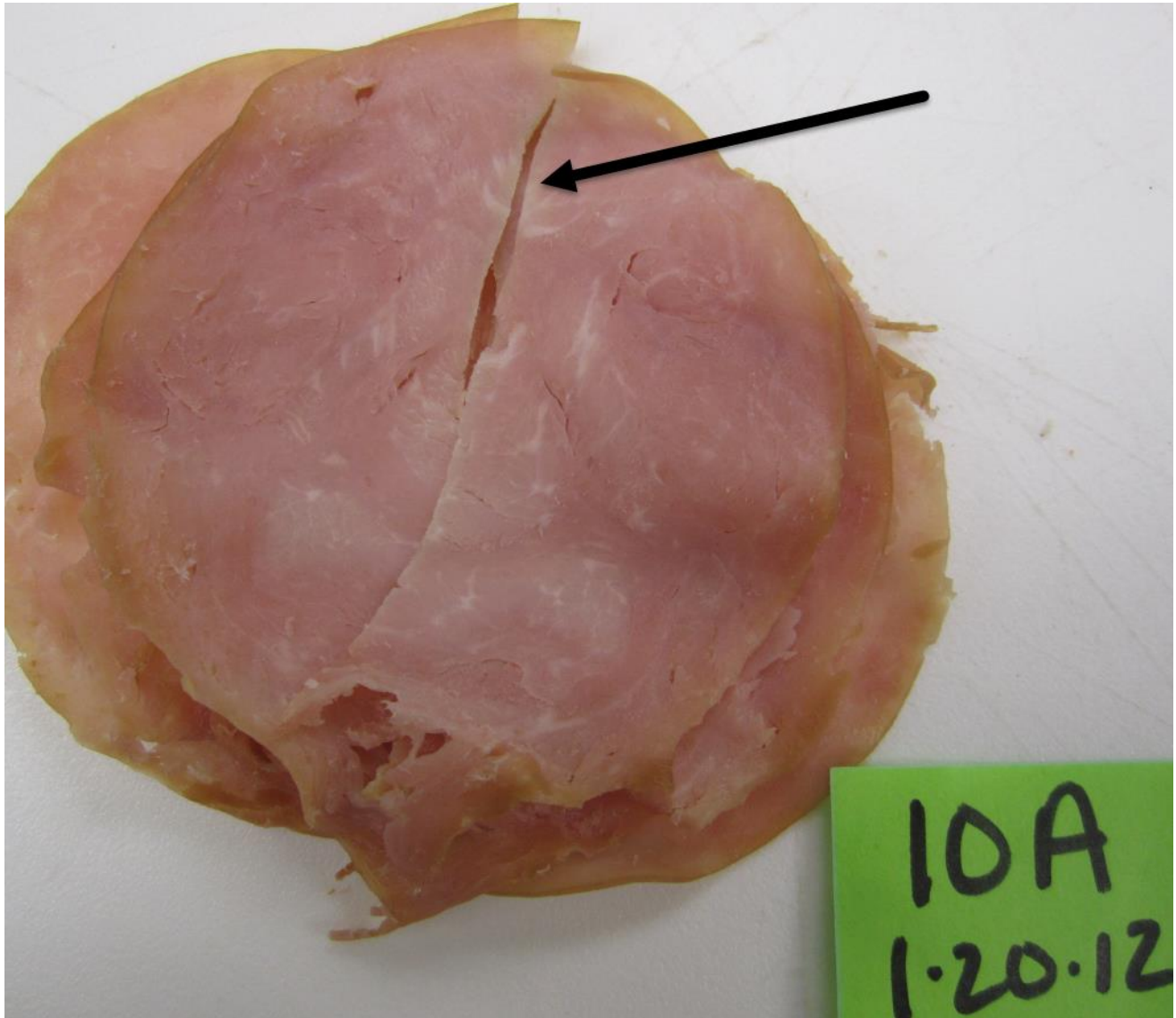




A.4: Day 13, appearance of the sandwiches in all coolers, of Test 1

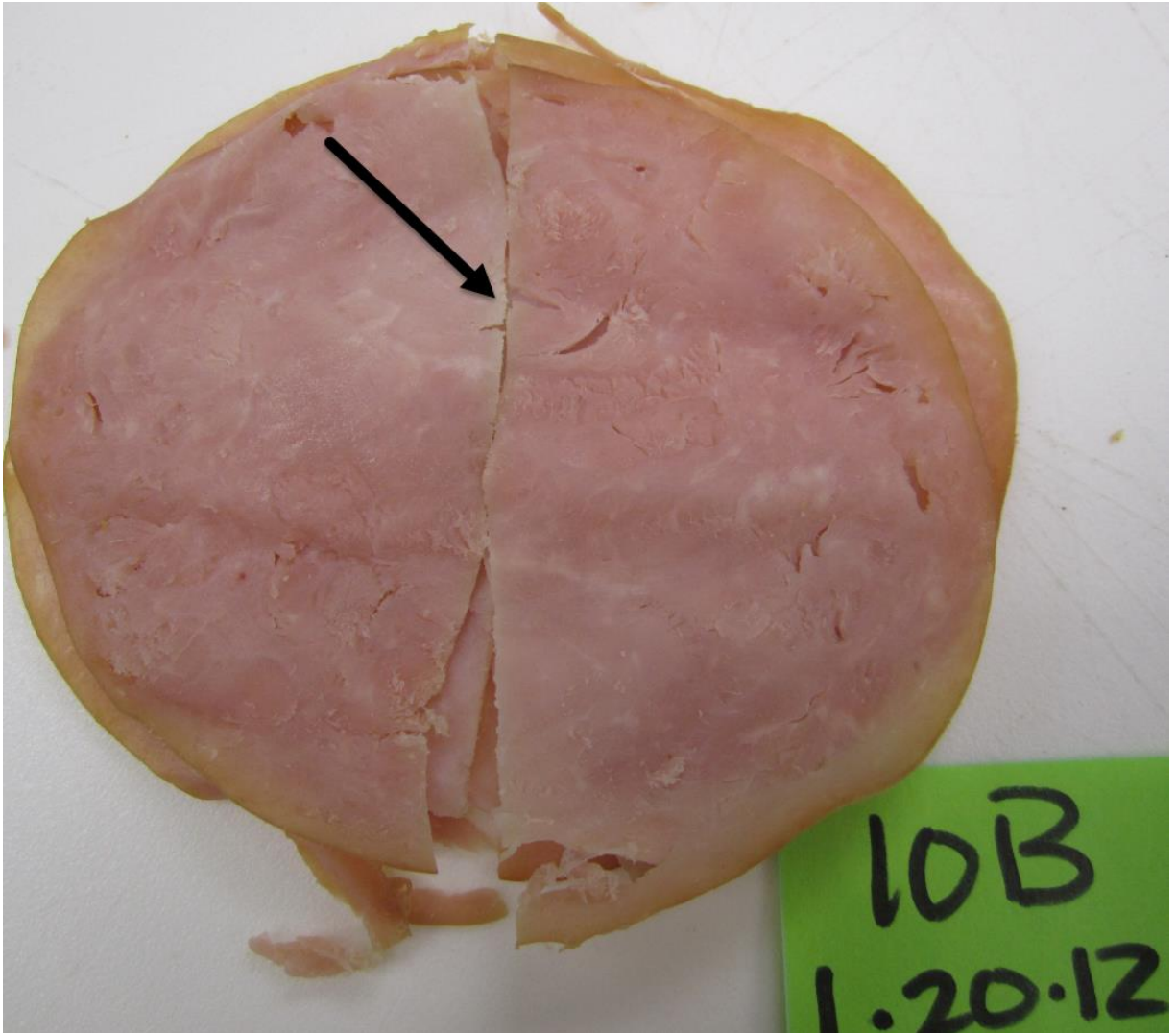


A.5.1: Day 14 of Test 1, Cooler A (open) (The area where the two half slices meet is the area exposed to light in the displayed wedge format) A: Open cooler B: LED light closed door cooler C: LED light closed door cooler D: Fluorescent bulb dark cooler E: Dark cooler – no light

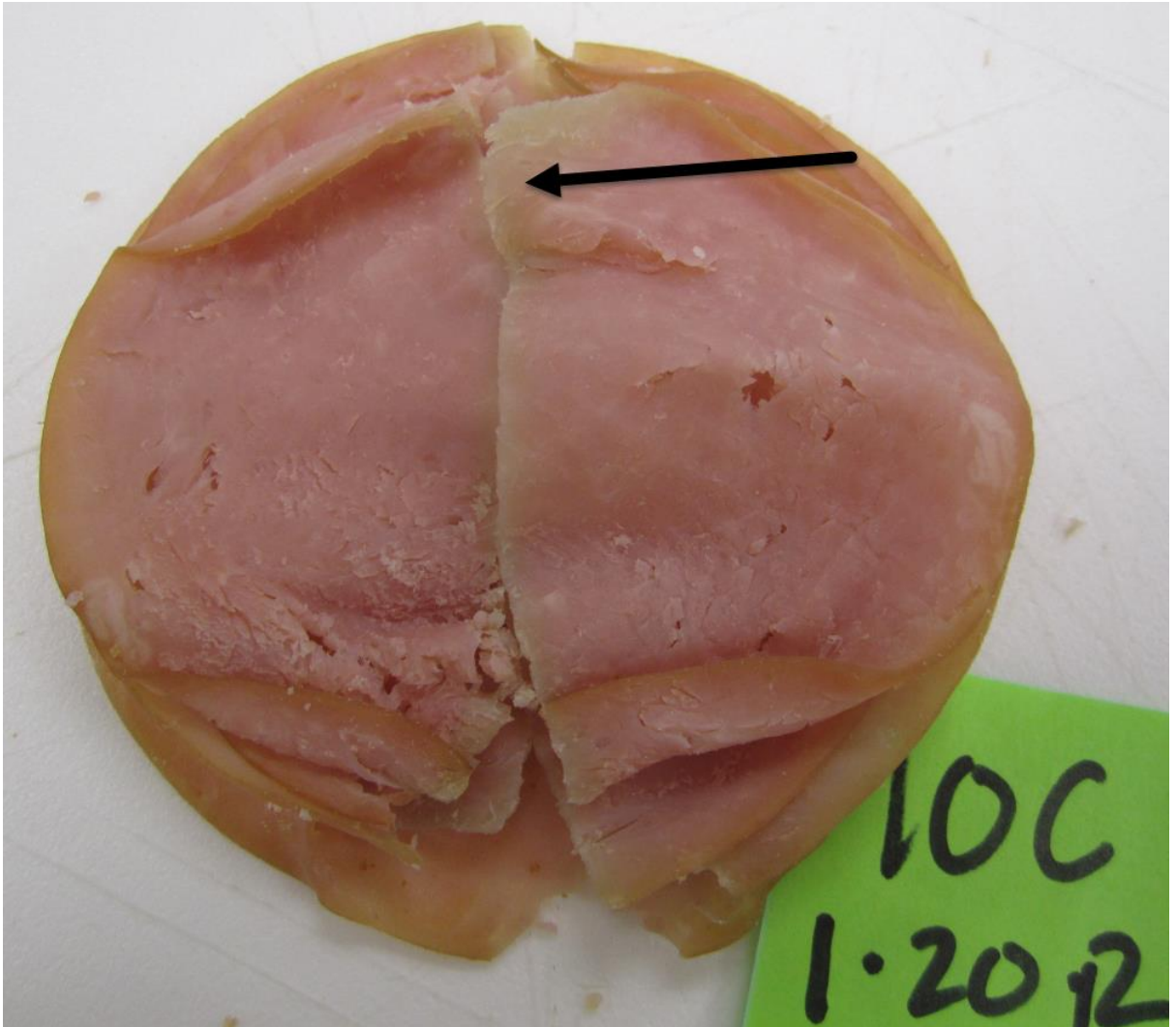




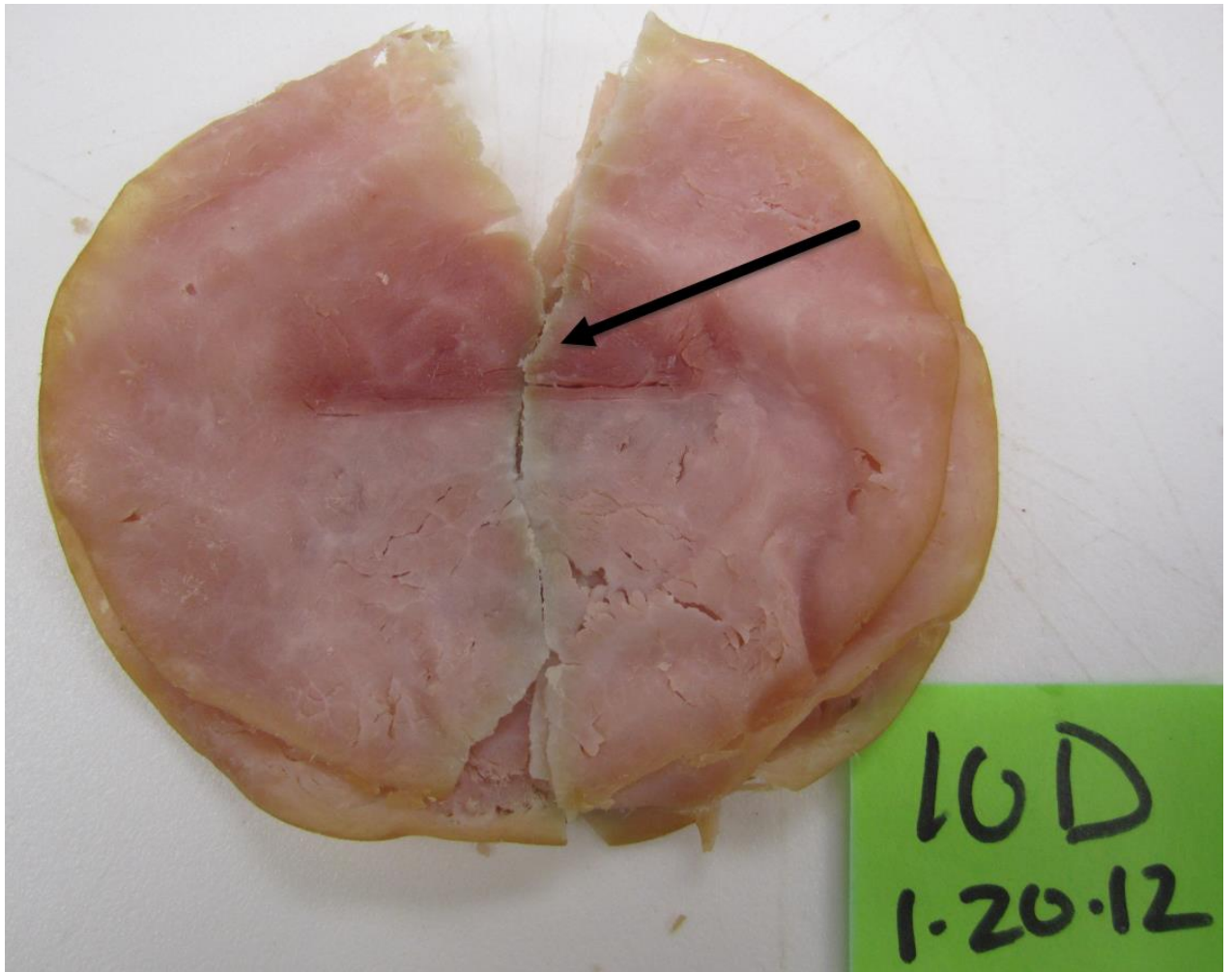
A.5.2: Day 14 of Test 1, Cooler B (LED)



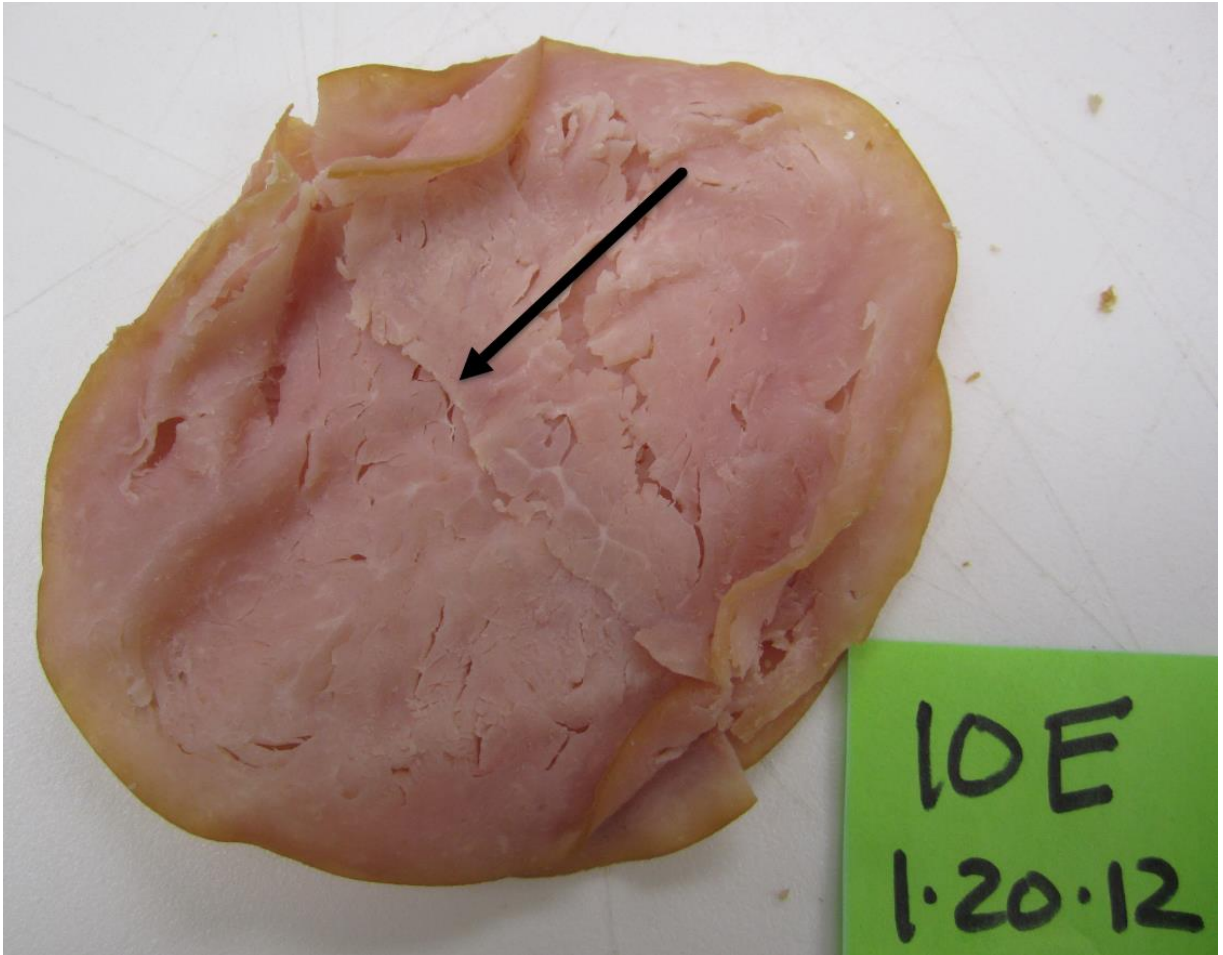
A.5.3: Day 14 of Test 1, Cooler C (LED)



A.5.4: Day 14 of Test 1, Cooler D (Fluorescent) Current model used by E.A. Sween/Deli Express

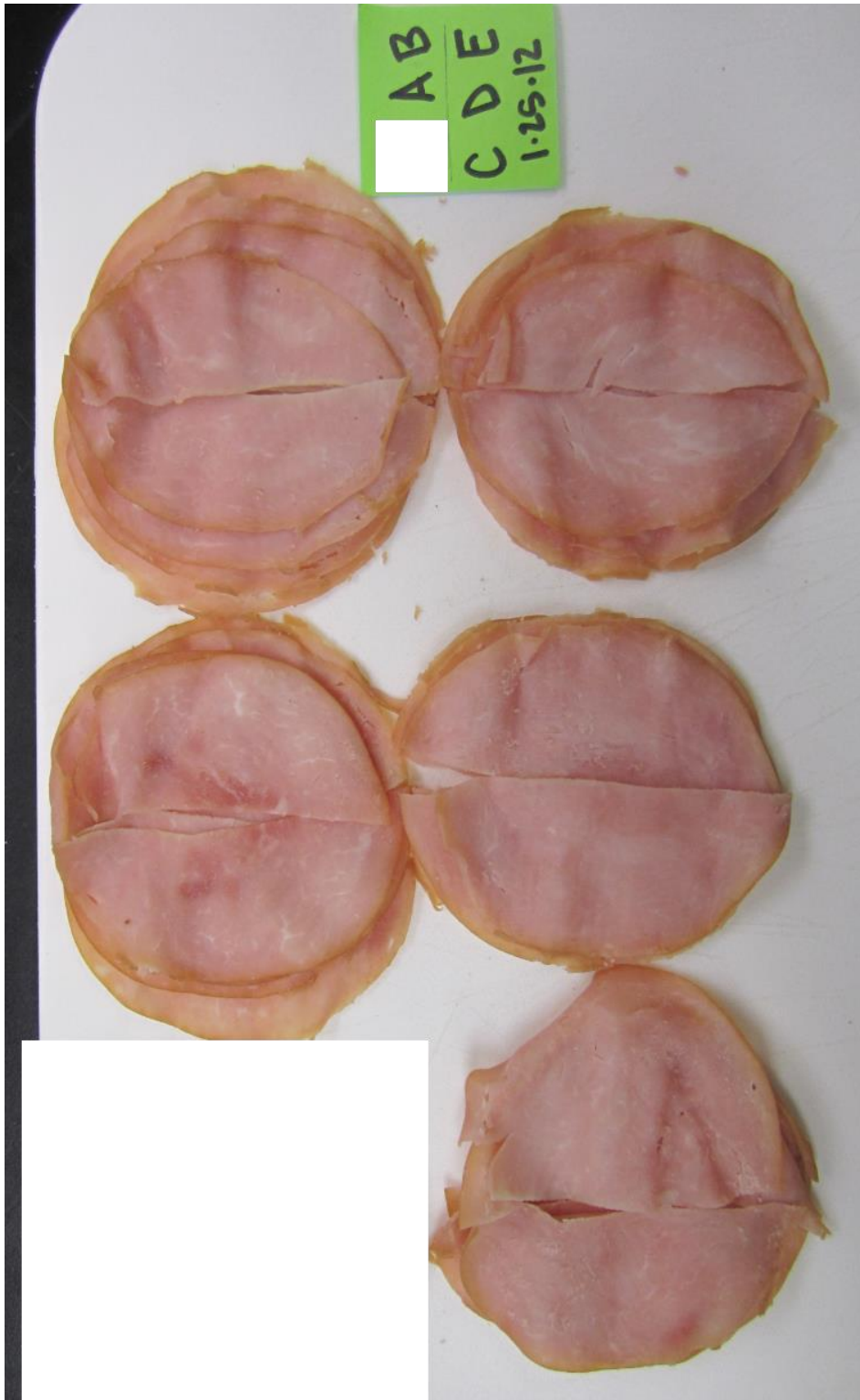


A.5.5: Day 14 of Test 1, Cooler E (dark)

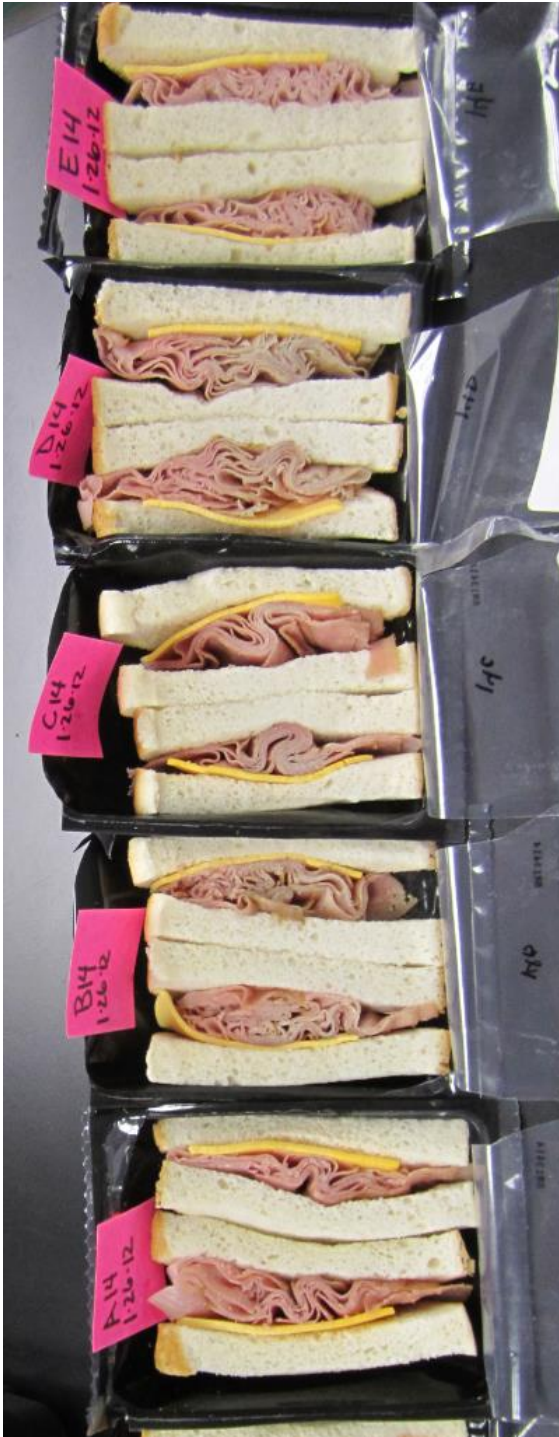




A.6: Day 19 of Test 1



A.7: Day 20 of Test 1





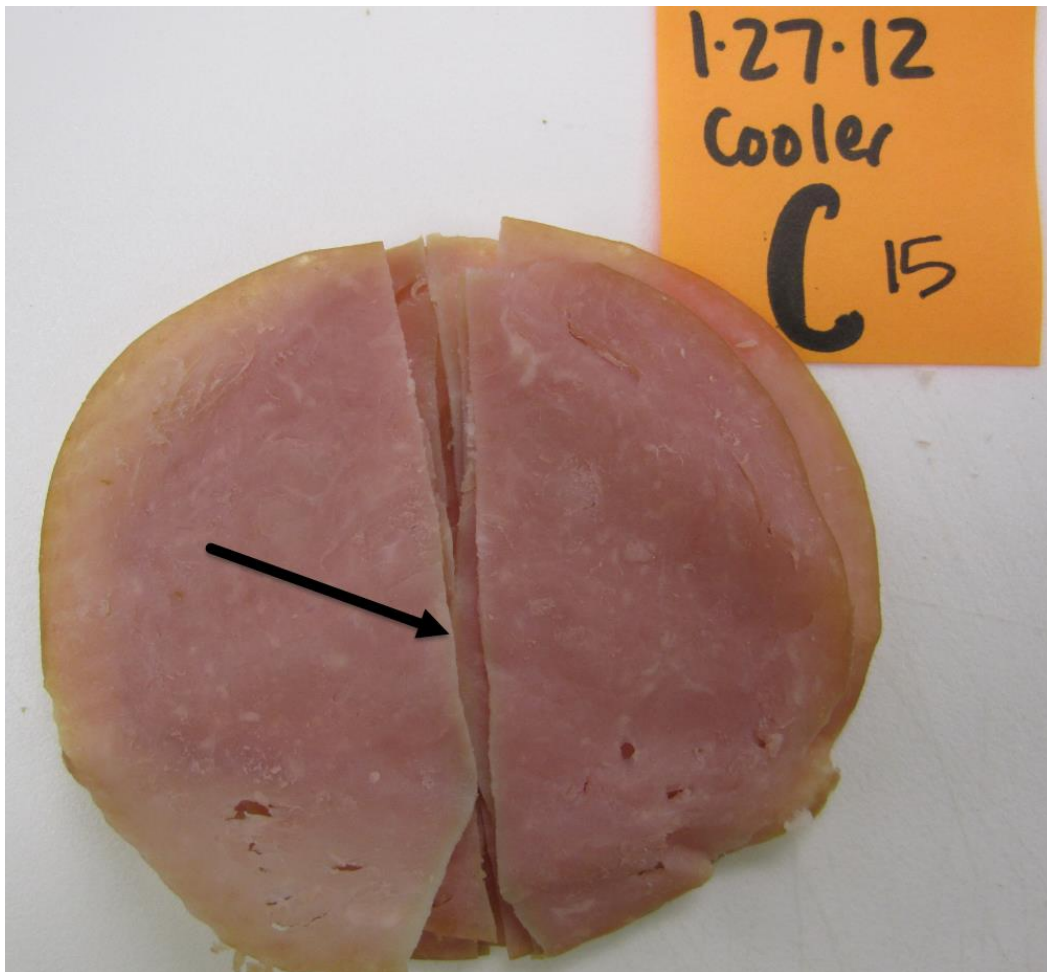
A.8.1: Day 21 of Test 1 (The area where the two half slices meet is the area exposed to light in the displayed wedge format) A: Open cooler B: LED light closed door cooler C: LED light closed door cooler D: Fluorescent bulb dark cooler E: Dark cooler – no light



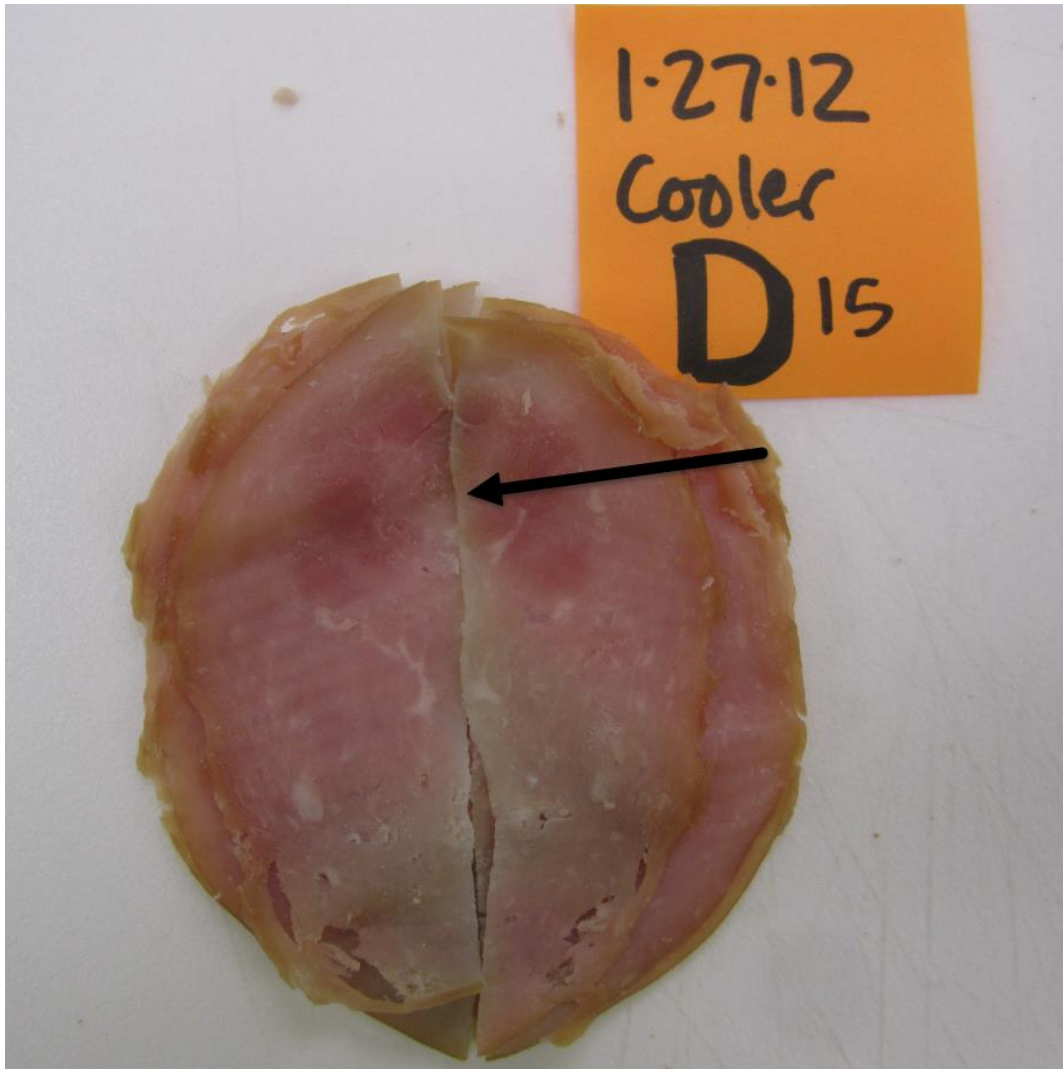
A.8.2: Day 21 of Test 1



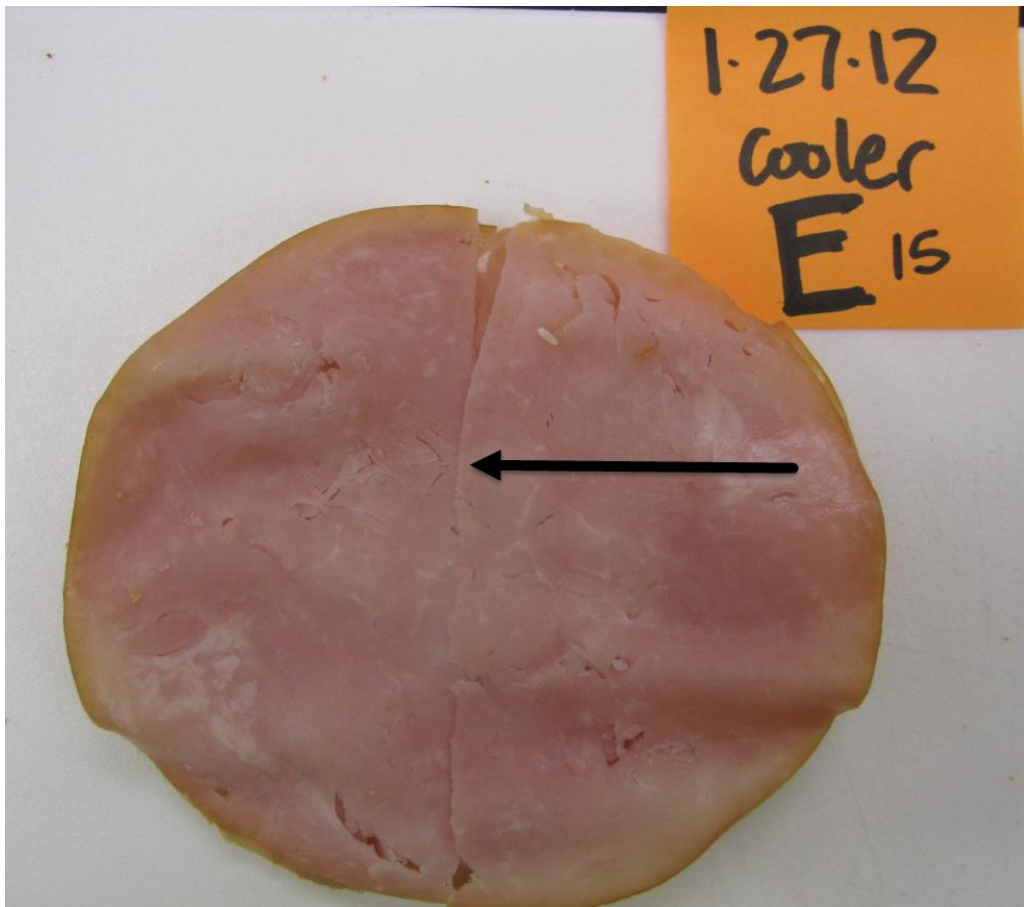
A.8.3: Day 21 of Test 1



A.8.4: Day 21 of Test 1

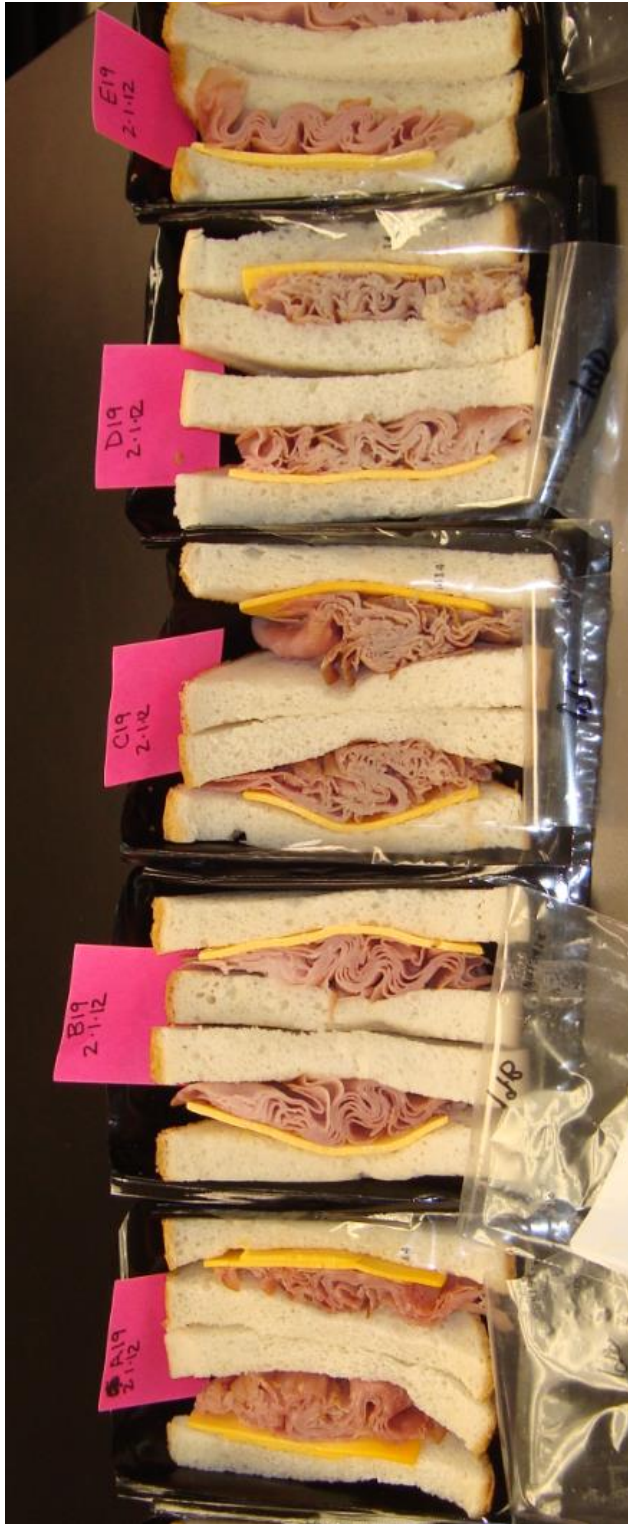


A.8.5: Day 21 of Test 1





A.9: Day 26 of Test 1



A.10: Day 31 of Test 1



A.11: Example of within sample color variation (top portion of the sandwich represents area under the label)

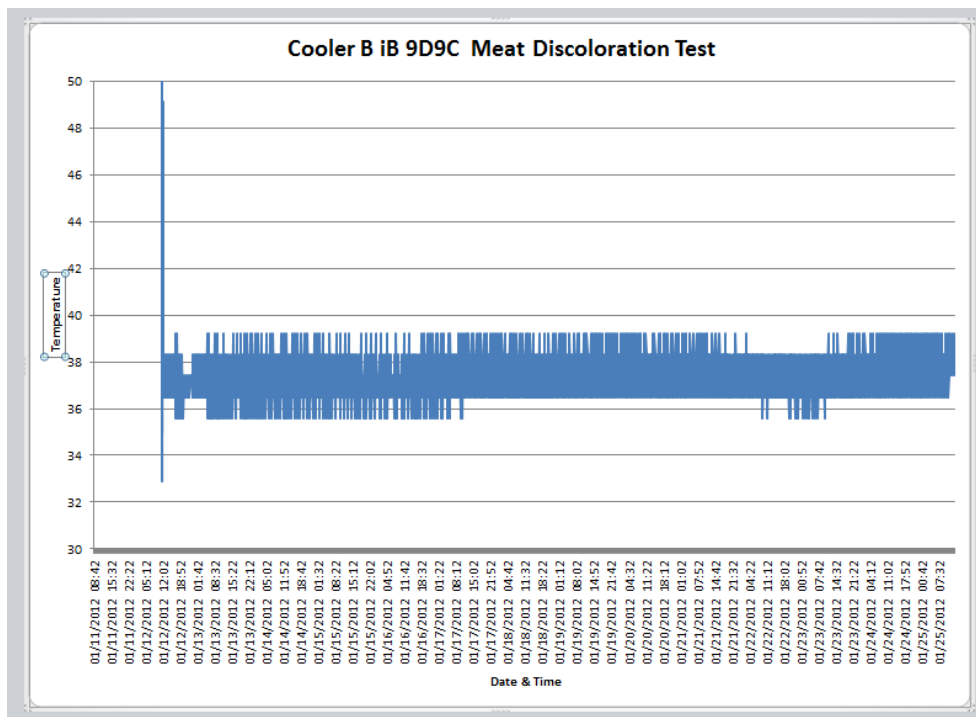
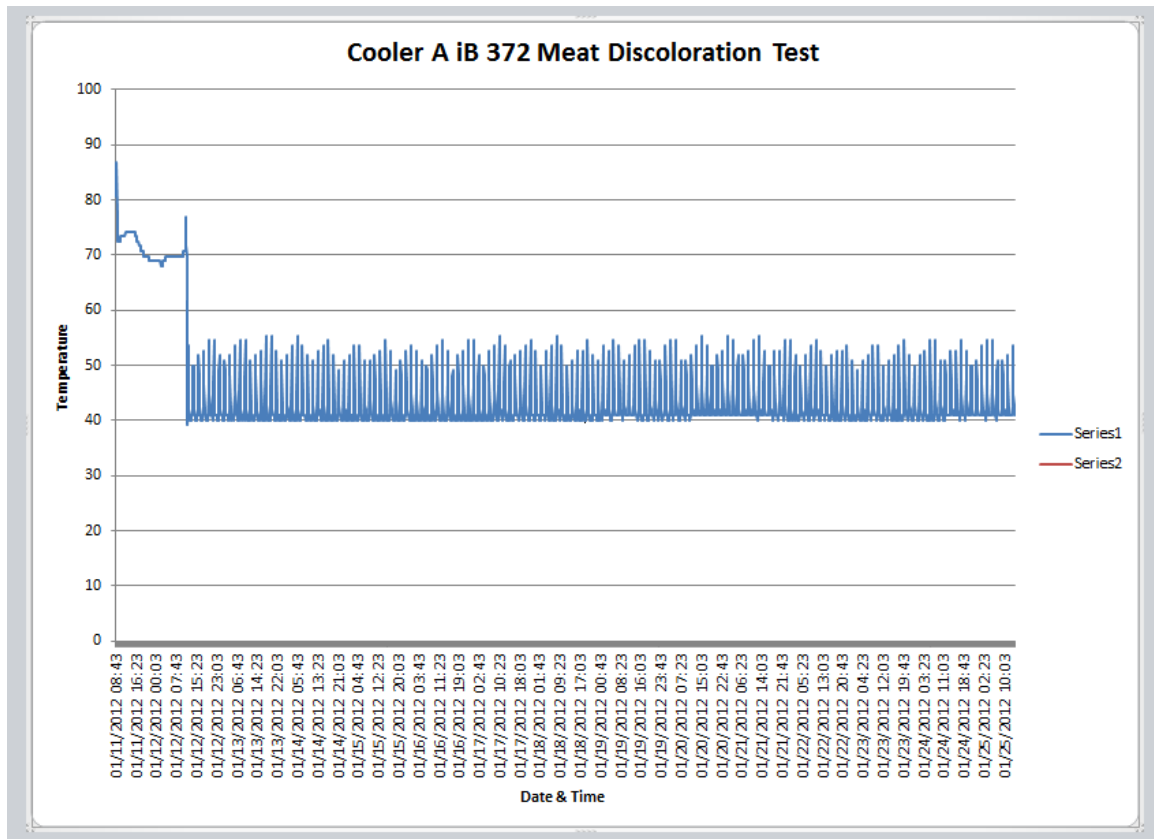


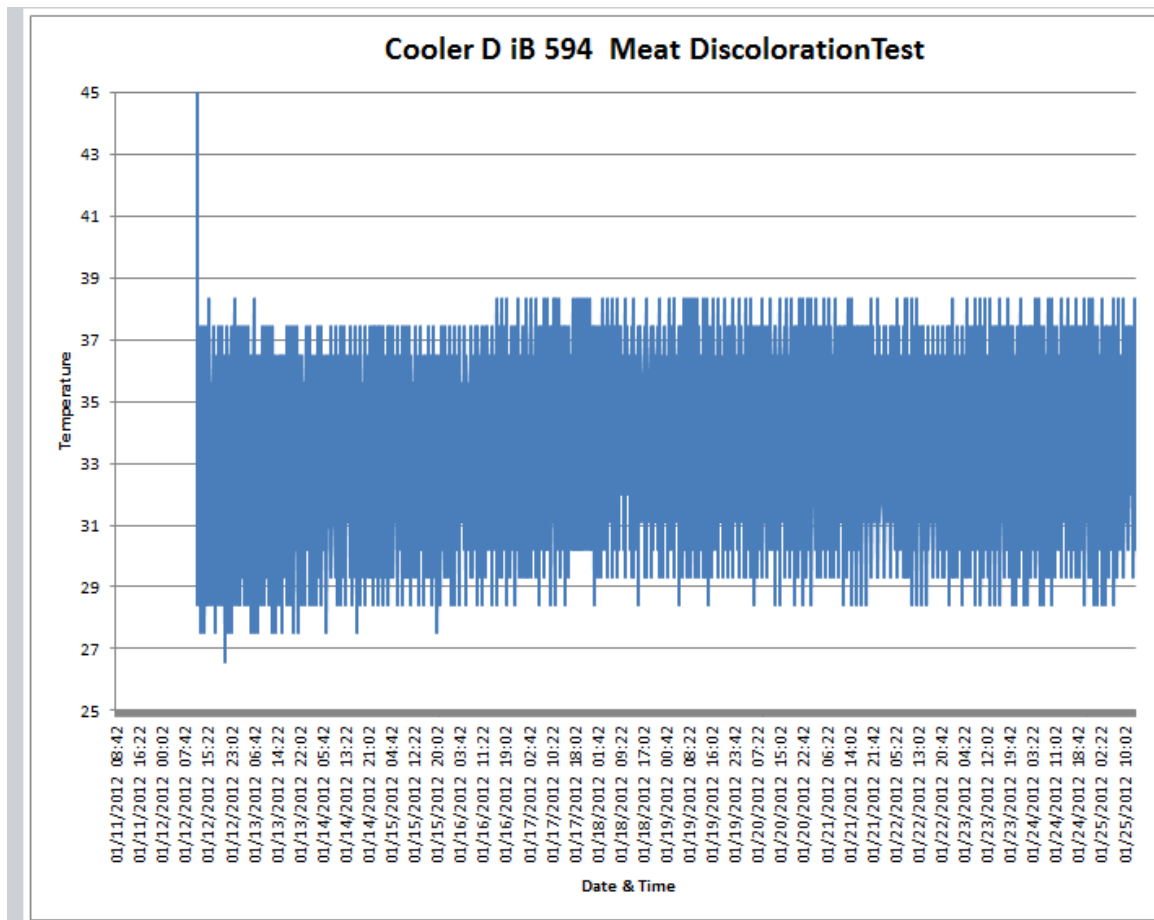
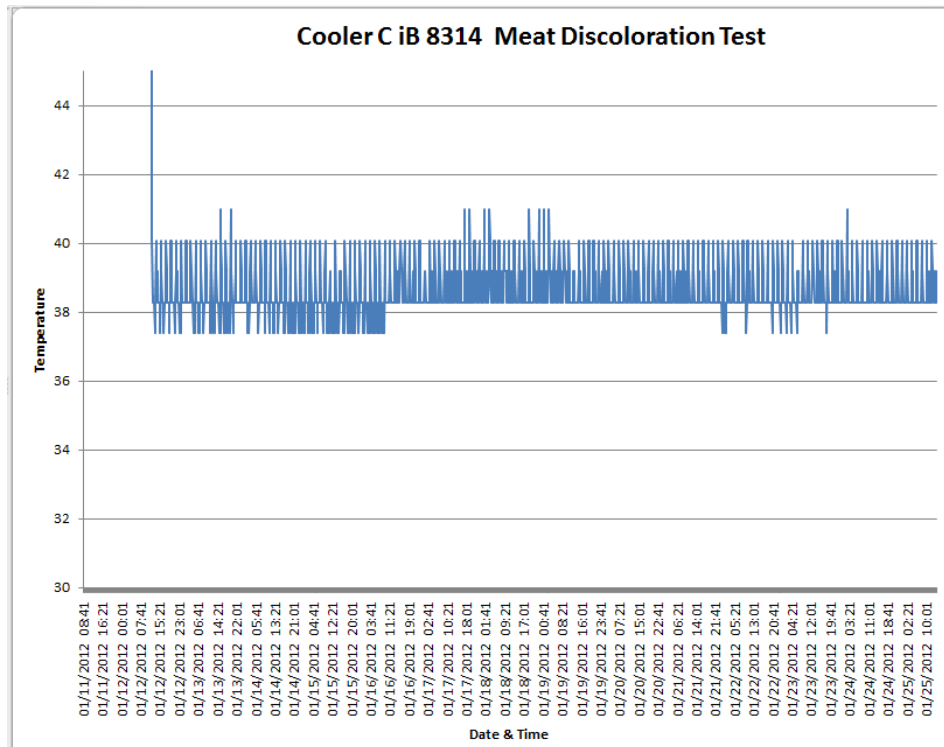


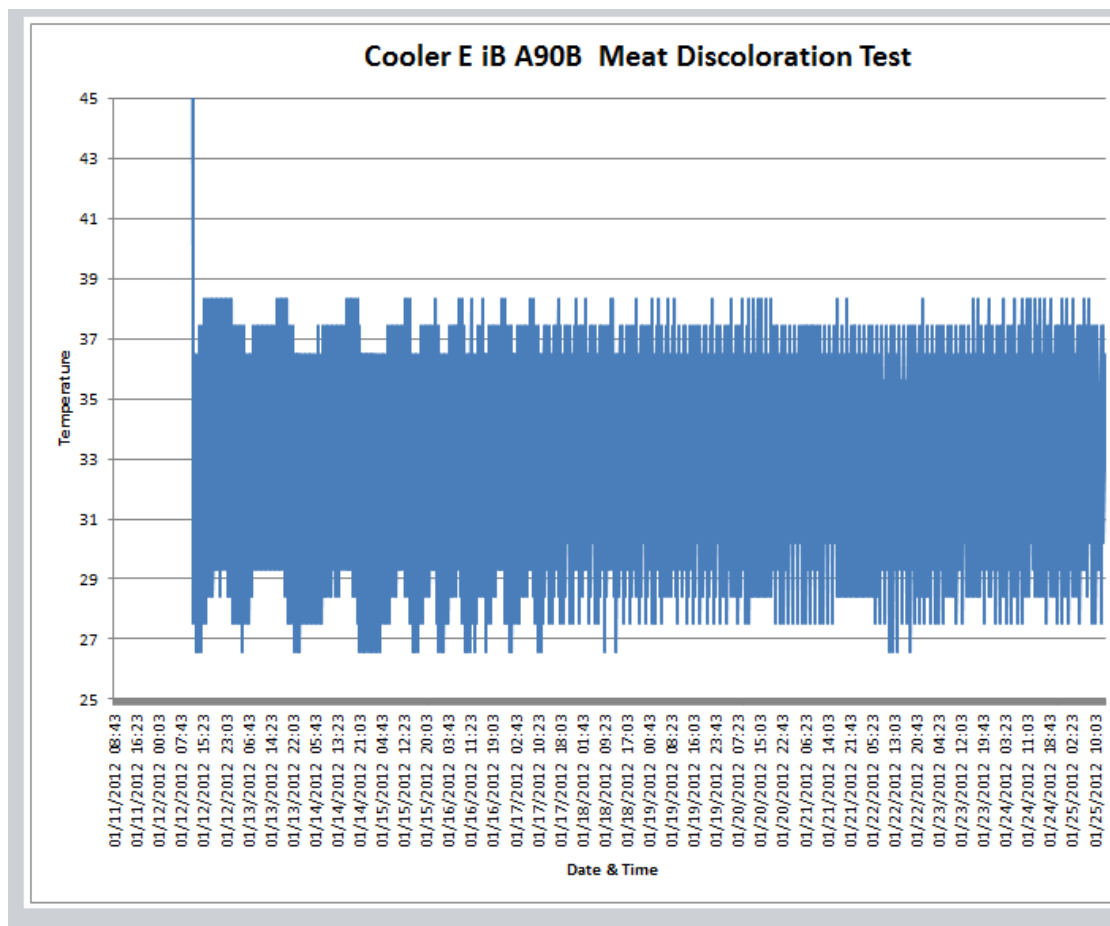
WEEK 1	Date	WEEK 2				WEEK 3				WEEK 4				Average 4 value Min 4 value Max 4 value								
	Measurement	Measurement				Measurement				Measurement												
	a <sup>1</sup>	a <sup>2</sup>	a <sup>3</sup>	a <sup>4</sup>	L <sup>1</sup>	L <sup>2</sup>	L <sup>3</sup>	L <sup>4</sup>	a <sup>1</sup>	a <sup>2</sup>	a <sup>3</sup>	a <sup>4</sup>	L <sup>1</sup>		L <sup>2</sup>	L <sup>3</sup>	L <sup>4</sup>					
	b <sup>1</sup>	b <sup>2</sup>	b <sup>3</sup>	b <sup>4</sup>	a <sup>1</sup>	a <sup>2</sup>	a <sup>3</sup>	a <sup>4</sup>	b <sup>1</sup>	b <sup>2</sup>	b <sup>3</sup>	b <sup>4</sup>	a <sup>1</sup>		a <sup>2</sup>	a <sup>3</sup>	a <sup>4</sup>					
	AE <sup>1</sup>	AE <sup>2</sup>	AE <sup>3</sup>	AE <sup>4</sup>	b <sup>1</sup>	b <sup>2</sup>	b <sup>3</sup>	b <sup>4</sup>	AE <sup>1</sup>	AE <sup>2</sup>	AE <sup>3</sup>	AE <sup>4</sup>	b <sup>1</sup>		b <sup>2</sup>	b <sup>3</sup>	b <sup>4</sup>					
MOCON - O2 (%)																	0.000	0.007	0.086	0.000	0.023	
Date Collected																	2/3/12	Day		28		
R&D Lab																	Cooler A	Cooler B	Cooler C	Cooler D	Cooler E	
MOCON - CO2 (%)																	23.3	22.4	22.9	22.0	22.4	
MOCON - O2 - (%)																	0.000	0.000	0.000	0.001	0.022	

WEEK 1	Date	Time	Measurement	1/9/2012										1/10/2012										1/11/2012										1/12/2012										1/13/2012										Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Day 72	Day 73	Day 74	Day 75	Day 76	Day 77	Day 78	Day 79	Day 80	Day 81	Day 82	Day 83	Day 84	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	Day 92	Day 93	Day 94	Day 95	Day 96	Day 97	Day 98	Day 99	Day 100	Day 101	Day 102	Day 103	Day 104	Day 105	Day 106	Day 107	Day 108	Day 109	Day 110	Day 111	Day 112	Day 113	Day 114	Day 115	Day 116	Day 117	Day 118	Day 119	Day 120	Day 121	Day 122	Day 123	Day 124	Day 125	Day 126	Day 127	Day 128	Day 129	Day 130	Day 131	Day 132	Day 133	Day 134	Day 135	Day 136	Day 137	Day 138	Day 139	Day 140	Day 141	Day 142	Day 143	Day 144	Day 145	Day 146	Day 147	Day 148	Day 149	Day 150	Day 151	Day 152	Day 153	Day 154	Day 155	Day 156	Day 157	Day 158	Day 159	Day 160	Day 161	Day 162	Day 163	Day 164	Day 165	Day 166	Day 167	Day 168	Day 169	Day 170	Day 171	Day 172	Day 173	Day 174	Day 175	Day 176	Day 177	Day 178	Day 179	Day 180	Day 181	Day 182	Day 183	Day 184	Day 185	Day 186	Day 187	Day 188	Day 189	Day 190	Day 191	Day 192	Day 193	Day 194	Day 195	Day 196	Day 197	Day 198	Day 199	Day 200	Day 201	Day 202	Day 203	Day 204	Day 205	Day 206	Day 207	Day 208	Day 209	Day 210	Day 211	Day 212	Day 213	Day 214	Day 215	Day 216	Day 217	Day 218	Day 219	Day 220	Day 221	Day 222	Day 223	Day 224	Day 225	Day 226	Day 227	Day 228	Day 229	Day 230	Day 231	Day 232	Day 233	Day 234	Day 235	Day 236	Day 237	Day 238	Day 239	Day 240	Day 241	Day 242	Day 243	Day 244	Day 245	Day 246	Day 247	Day 248	Day 249	Day 250	Day 251	Day 252	Day 253	Day 254	Day 255	Day 256	Day 257	Day 258	Day 259	Day 260	Day 261	Day 262	Day 263	Day 264	Day 265	Day 266	Day 267	Day 268	Day 269	Day 270	Day 271	Day 272	Day 273	Day 274	Day 275	Day 276	Day 277	Day 278	Day 279	Day 280	Day 281	Day 282	Day 283	Day 284	Day 285	Day 286	Day 287	Day 288	Day 289	Day 290	Day 291	Day 292	Day 293	Day 294	Day 295	Day 296	Day 297	Day 298	Day 299	Day 300	Day 301	Day 302	Day 303	Day 304	Day 305	Day 306	Day 307	Day 308	Day 309	Day 310	Day 311	Day 312	Day 313	Day 314	Day 315	Day 316	Day 317	Day 318	Day 319	Day 320	Day 321	Day 322	Day 323	Day 324	Day 325	Day 326	Day 327	Day 328	Day 329	Day 330	Day 331	Day 332	Day 333	Day 334	Day 335	Day 336	Day 337	Day 338	Day 339	Day 340	Day 341	Day 342	Day 343	Day 344	Day 345	Day 346	Day 347	Day 348	Day 349	Day 350	Day 351	Day 352	Day 353	Day 354	Day 355	Day 356	Day 357	Day 358	Day 359	Day 360	Day 361	Day 362	Day 363	Day 364	Day 365	Day 366	Day 367	Day 368	Day 369	Day 370	Day 371	Day 372	Day 373	Day 374	Day 375	Day 376	Day 377	Day 378	Day 379	Day 380	Day 381	Day 382	Day 383	Day 384	Day 385	Day 386	Day 387	Day 388	Day 389	Day 390	Day 391	Day 392	Day 393	Day 394	Day 395	Day 396	Day 397	Day 398	Day 399	Day 400	Day 401	Day 402	Day 403	Day 404	Day 405	Day 406	Day 407	Day 408	Day 409	Day 410	Day 411	Day 412	Day 413	Day 414	Day 415	Day 416	Day 417	Day 418	Day 419	Day 420	Day 421	Day 422	Day 423	Day 424	Day 425	Day 426	Day 427	Day 428	Day 429	Day 430	Day 431	Day 432	Day 433	Day 434	Day 435	Day 436	Day 437	Day 438	Day 439	Day 440	Day 441	Day 442	Day 443	Day 444	Day 445	Day 446	Day 447	Day 448	Day 449	Day 450	Day 451	Day 452	Day 453	Day 454	Day 455	Day 456	Day 457	Day 458	Day 459	Day 460	Day 461	Day 462	Day 463	Day 464	Day 465	Day 466	Day 467	Day 468	Day 469	Day 470	Day 471	Day 472	Day 473	Day 474	Day 475	Day 476	Day 477	Day 478	Day 479	Day 480	Day 481	Day 482	Day 483	Day 484	Day 485	Day 486	Day 487	Day 488	Day 489	Day 490	Day 491	Day 492	Day 493	Day 494	Day 495	Day 496	Day 497	Day 498	Day 499	Day 500	Day 501	Day 502	Day 503	Day 504	Day 505	Day 506	Day 507	Day 508	Day 509	Day 510	Day 511	Day 512	Day 513	Day 514	Day 515	Day 516	Day 517	Day 518	Day 519	Day 520	Day 521	Day 522	Day 523	Day 524	Day 525	Day 526	Day 527	Day 528	Day 529	Day 530	Day 531	Day 532	Day 533	Day 534	Day 535	Day 536	Day 537	Day 538	Day 539	Day 540	Day 541	Day 542	Day 543	Day 544	Day 545	Day 546	Day 547	Day 548	Day 549	Day 550	Day 551	Day 552	Day 553	Day 554	Day 555	Day 556	Day 557	Day 558	Day 559	Day 560	Day 561	Day 562	Day 563	Day 564	Day 565	Day 566	Day 567	Day 568	Day 569	Day 570	Day 571	Day 572	Day 573	Day 574	Day 575	Day 576	Day 577	Day 578	Day 579	Day 580	Day 581	Day 582	Day 583	Day 584	Day 585	Day 586	Day 587	Day 588	Day 589	Day 590	Day 591	Day 592	Day 593	Day 594	Day 595	Day 596	Day 597	Day 598	Day 599	Day 600	Day 601	Day 602	Day 603	Day 604	Day 605	Day 606	Day 607	Day 608	Day 609	Day 610	Day 611	Day 612	Day 613	Day 614	Day 615	Day 616	Day 617	Day 618	Day 619	Day 620	Day 621	Day 622	Day 623	Day 624	Day 625	Day 626	Day 627	Day 628	Day 629	Day 630	Day 631	Day 632	Day 633	Day 634	Day 635	Day 636	Day 637	Day 638	Day 639	Day 640	Day 641	Day 642	Day 643	Day 644	Day 645	Day 646	Day 647	Day 648	Day 649	Day 650	Day 651	Day 652	Day 653	Day 654	Day 655	Day 656	Day 657	Day 658	Day 659	Day 660	Day 661	Day 662	Day 663	Day 664	Day 665	Day 666	Day 667	Day 668	Day 669	Day 670	Day 671	Day 672	Day 673	Day 674	Day 675	Day 676	Day 677	Day 678	Day 679	Day 680	Day 681	Day 682	Day 683	Day 684	Day 685	Day 686	Day 687	Day 688	Day 689	Day 690	Day 691	Day 692	Day 693	Day 694	Day 695	Day 696	Day 697	Day 698	Day 699	Day 700	Day 701	Day 702	Day 703	Day 704	Day 705	Day 706	Day 707	Day 708	Day 709	Day 710	Day 711	Day 712	Day 713	Day 714	Day 715	Day 716	Day 717	Day 718	Day 719	Day 720	Day 721	Day 722	Day 723	Day 724	Day 725	Day 726	Day 727	Day 728	Day 729	Day 730	Day 731	Day 732	Day 733	Day 734	Day 735	Day 736	Day 737	Day 738	Day 739	Day 740	Day 741	Day 742	Day 743	Day 744	Day 745	Day 746	Day 747	Day 748	Day 749	Day 750	Day 751	Day 752	Day 753	Day 754	Day 755	Day 756	Day 757	Day 758	Day 759	Day 760	Day 761	Day 762	Day 763	Day 764	Day 765	Day 766	Day 767	Day 768	Day 769	Day 770	Day 771	Day 772	Day 773	Day 774	Day 775	Day 776	Day 777	Day 778	Day 779	Day 780	Day 781	Day 782	Day 783	Day 784	Day 785	Day 786	Day 787	Day 788	Day 789	Day 790	Day 791	Day 792	Day 793	Day 794	Day 795	Day 796	Day 797	Day 798	Day 799	Day 800	Day 801	Day 802	Day 803	Day 804	Day 805	Day 806	Day 807	Day 808	Day 809	Day 810	Day 811	Day 812	Day 813	Day 814	Day 815	Day 816	Day 817	Day 818	Day 819	Day 820	Day 821	Day 822	Day 823	Day 824	Day 825	Day 826	Day 827	Day 828	Day 829	Day 830	Day 831	Day 832	Day 833	Day 834	Day 835	Day 836	Day 837	Day 838	Day 839	Day 840	Day 841	Day 842	Day 843	Day 844	Day 845	Day 846	Day 847	Day 848	Day 849	Day 850	Day 851	Day 852	Day 853	Day 854	Day 855	Day 856	Day 857	Day 858	Day 859	Day 860	Day 861	Day 862	Day 863	Day 864	Day 865	Day 866	Day 867	Day 868	Day 869	Day 870	Day 871	Day 872	Day 873	Day 874	Day 875	Day 876	Day 877	Day 878	Day 879	Day 880	Day 881	Day 882	Day 883	Day 884	Day 885	Day 886	Day 887	Day 888	Day 889	Day 890	Day 891	Day 892	Day 893	Day 894	Day 895	Day 896	Day 897	Day 898	Day 899	Day 900	Day 901	Day 902	Day 903	Day 904	Day 905	Day 906	Day 907	Day 908	Day 909	Day 910	Day 911	Day 912	Day 913	Day 914	Day 915	Day 916	Day 917	Day 918	Day 919	Day 920	Day 921	Day 922	Day 923	Day 924	Day 925	Day 926	Day 927	Day 928	Day 929	Day 930	Day 931	Day 932	Day 933	Day 934	Day 935	Day 936	Day 937	Day 938	Day 939	Day 940	Day 941	Day 942	Day 943	Day 944	Day 945	Day 946	Day 947	Day 948	Day 949	Day 950	Day 951	Day 952	Day 953	Day 954	Day 955	Day 956</
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### A.13 – Cooler temperature raw data Test 1 (in Fahrenheit)







B.1 Raw data for Carbon dioxide and oxygen percentages in the headspace at the time of color measurement.

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B.2 Visual appearance of the control sample compared to the 5.0% test sample at day 1. Discoloration on the 5.0% sample is already detectable. Discoloration is not evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9							
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27	
		C28	C29	C30	C31	C32	C33	C34	
		C35	C36	C37	C38	C39	C40 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		
Cooler B									
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7	
		C8	C9	C10	C11	C12	C13	C14	
		C15	C16	C17	C18	C19	C20 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		

Cooler location



B.3 Visual appearance of the control sample compared to the 5.0% test sample at day 6. Discoloration on the 5.0% sample is already detectable. The area covered by the label allows for another area of comparison. Discoloration is not evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

B.4 Visual appearance of the control sample compared to the 5.0% test sample at day 11. Discoloration on the 5.0% sample is already detectable. The area covered by the label allows for another area of comparison. Discoloration is evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M2/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	



B.5 Visual appearance of the control sample compared to the 5.0% test sample at day 13. Discoloration on the 5.0% sample is already detectable. The area covered by the label allows for another area of comparison. Discoloration is evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

B.6 Visual appearance of the control sample compared to the 5.0% test sample at day 18. Discoloration on the 5.0% sample is already detectable. The area covered by the label allows for another area of comparison. Discoloration is evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	



B.7 Visual appearance of the control sample compared to the 5.0% test sample at day 22. Discoloration on the 5.0% sample is already detectable. The area covered by the label allows for another area of comparison. Discoloration is evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

B.8 Visual appearance of the control sample compared to the 5.0% test sample at day 29. Discoloration on the 5.0% sample is already detectable. The area covered by the label allows for another area of comparison. Discoloration is evident on the 0.0% sample compared to the control. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A		M3/19 F3/16 W3/14 M3/12 F3/9						
Test 5	Top - 1	C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	
Cooler B								
Test 0	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

## Appendix B – Test 2b UV (Ultraviolet) barrier film blocking at 380 nm compared to non-UV blocking packaging

B.9 Raw data for Carbon dioxide and oxygen percentages in the headspace at the time of color measurement

WEEK 1				WEEK 2				WEEK 3				WEEK 4			
day	Measurement	CO2	O2	day	Measurement	CO2	O2	day	Measurement	CO2	O2	day	Measurement	CO2	O2
1	Control UV 47	22.40	0.01	11	Control UV43	19.90	0.05	18	Control UV 52	19.80	0.00	25	Control UV 59	19.70	0.00
4	Control UV 46	20.90	0.02	13	Control UV 54	20.10	0.00	20	Control UV 51	19.80	0.01	27	Control UV 58	19.30	0.00
8	Control UV 44	20.30	0.04	15	Control UV 53	20.40	0.01	22	Control UV 50	19.20	0.05	29	Control UV 57	22.20	0.03
1	Test UV #6	21.10	0.11	11	Test UV #14	18.20	0.00	18	Test UV #11	18.60	0.32	25	Test UV #19	19.70	0.00
4	Test UV #5	20.80	0.08	13	Test UV #13	19.80	0.00	20	Test UV #10	20.00	0.02	27	Test UV #18	18.50	0.02
8	Test UV #3	18.70	0.09	15	Test UV #12	19.20	0.21	22	Test UV #20	19.10	0.00	29	Test UV #17	22.10	0.04

B.10 Visual appearance of the control sample compared to the UV test sample at day 1. Discoloration is not evident on the 0.0% sample compared to the control.



Cooler C									
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47	
		C48	C49	C50	C51	C52	C53	C54	
		C55	C56	C57	C58	C59	C60 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		



B.11 Visual appearance of the control sample compared to the UV test sample at day 6. Discoloration is not evident on the 0.0% sample compared to the control.



Cooler C								
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47
		C48	C49	C50	C51	C52	C53	C54
		C55	C56	C57	C58	C59	C60 Pic	
		1	2	3	4	5	6	7 Pic
		8	9	10	11	12	13	14
	Bottom shelf - 6	15	16	17	18	19	20	

B.12 Visual appearance of the control sample compared to the UV test sample at day 11. Discoloration is detectable on the 0.0% sample compared to the control.



Cooler C									
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47	
		C48	C49	C50	C51	C52	C53	C54	
		C55	C56	C57	C58	C59	C60 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		



B.13 Visual appearance of the control sample compared to the UV test sample at day 13. Discoloration is evident on both control and test.



Cooler C									
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47	
		C48	C49	C50	C51	C52	C53	C54	
		C55	C56	C57	C58	C59	C60 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		



B.14 Visual appearance of the control sample compared to the UV test sample at day 18. Discoloration is evident on both control and test.



Cooler C									
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47	
		C48	C49	C50	C51	C52	C53	C54	
		C55	C56	C57	C58	C59	C60 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		

B.15 Visual appearance of the control sample compared to the UV test sample at day 22. Discoloration is evident on the UV sample compared to the control.



Cooler C									
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47	
		C48	C49	C50	C51	C52	C53	C54	
		C55	C56	C57	C58	C59	C60 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		

B.16 Visual appearance of the control sample compared to the UV test sample at day 29. Discoloration is not evident on either.



Cooler C									
Test UV	Top - 1	C41	C42	C43	C44	C45	C46	C47	
		C48	C49	C50	C51	C52	C53	C54	
		C55	C56	C57	C58	C59	C60 Pic		
		1	2	3	4	5	6	7 Pic	
		8	9	10	11	12	13	14	
	Bottom shelf - 6	15	16	17	18	19	20		

# B.17 – raw $L^*a^*b^*$ data for tests 2a and 2b

WEEK 1 March 8-16, 2012						
Date Collected	3/8/12		Day	1		
POS Building	Cooler A		Cooler B		Cooler C	
Sample Number	Control 27	Test 5 #6	Control 7	Test #6	Control 47	Test UV #6
Cooler Observations						
R&D Lab	Control 27	Test 5 #6	Control 7	Test #6	Control 47	Test UV #6
MOCON - CO2 (%)	22.0	15.0	22.6	20.7	22.4	21.1
MOCON - O2 (%)	0.08	5.19	0.06	0.04	0.01	0.11
	Control 7	Test 5 #6	Control 27	Test 0 #6	Control 47	Test UV #6
L* (1)	58.81	58.54	57.59	60.13	60.22	59.76
a* (1)	19.08	13.54	18.96	18.56	18.13	18.89
b* (1)	7.39	10.90	7.15	8.35	7.39	7.91
L* (2)	55.94	60.30	58.36	61.39	59.86	59.55
a* (2)	20.07	14.03	18.96	17.69	18.70	17.95
b* (2)	6.70	9.54	7.14	7.30	7.35	8.59
L* (3)	55.75	59.81	57.01	62.45	60.15	60.39
a* (3)	19.87	15.48	20.11	17.07	18.73	17.66
b* (3)	6.62	8.41	7.04	7.51	8.27	8.16
L* AVERAGE	56.83	59.55	57.65	61.32	60.08	59.90
a* AVERAGE	19.67	14.35	19.34	17.77	18.52	18.17
b* AVERAGE	6.90	9.62	7.11	7.72	7.67	8.22
Date Collected	3/12/12		Day	4		
POS Building	Cooler A		Cooler B		Cooler C	
Sample Number	Control 26	Test 5 #5	Control 6	Test 0 #5	Control 46	Test UV #5
Cooler Observations	Gray, discolored					
R&D Lab	Control 26	Test 5 #5	Control 6	Test 0 #5	Control 46	Test UV #5
MOCON - CO2 (%)	21.0	14.3	21.1	21.0	20.9	20.8
MOCON - O2 (%)	0.07	4.50	0.06	0.03	0.02	0.08
	Control 26	Test 5 #5	Control 6	Test 0 #5	Control 46	Test UV #5
L* (1)	60.99	57.95	59.80	60.30	61.27	61.79
a* (1)	17.12	14.85	18.49	18.37	15.94	15.20
b* (1)	8.71	9.15	7.77	8.83	9.45	7.22
L* (2)	61.30	59.80	59.14	60.81	60.16	65.35
a* (2)	17.50	15.87	19.52	18.51	17.89	14.13
b* (2)	7.36	7.64	7.16	8.11	7.68	7.76
L* (3)	61.37	57.46	58.37	60.20	58.13	62.15
a* (3)	17.59	15.67	19.40	18.43	19.27	15.02
b* (3)	6.84	9.28	7.67	8.07	8.24	8.58
L* AVERAGE	61.22	58.40	59.10	60.44	59.85	63.10
a* AVERAGE	17.40	15.46	19.14	18.44	17.70	14.78
b* AVERAGE	7.64	8.69	7.53	8.34	8.46	7.85
Date Collected	3/14/12		Day	6		
POS Building	Cooler A		Cooler B		Cooler C	
Sample Number	Control 25	Test 5 #4	Control 5	Test 0 #4	Control 45	Test UV #4
Cooler Observations						
R&D Lab	Control 25	Test 5 #4	Control 5	Test 0 #4	Control 45	Test UV #4
MOCON - CO2 (%)						
MOCON - O2 (%)						
	Control 25	Test 5 #4	Control 5	Test 0 #4	Control 45	Test UV #4
L* (1)	58.18	59.76	58.89	59.08	61.58	60.15
a* (1)	19.11	13.30	18.20	17.89	15.49	18.84
b* (1)	8.41	9.47	6.84	8.30	7.27	8.26
L* (2)	59.65	60.35	58.25	59.64	61.66	61.16
a* (2)	19.49	13.21	19.31	18.38	15.86	18.63
b* (2)	8.11	9.53	7.63	7.60	7.61	8.00
L* (3)	59.33	59.73	58.39	59.20	60.34	60.57
a* (3)	19.35	13.94	19.73	18.05	16.11	17.96
b* (3)	8.01	9.24	8.18	7.98	7.59	8.09
ΔL* (3)	0.22	0.62	-0.04	0.77	-1.41	-1.17
Δa* (3)	-0.51	-5.92	0.44	-1.23	0.22	2.06
Δb* (3)	-0.80	0.43	0.70	0.50	0.02	0.52
ΔE* (3)	0.97	5.97	0.83	1.54	1.43	2.43
L* AVERAGE	59.05	59.95	58.51	59.31	61.19	60.63
a* AVERAGE	19.32	13.48	19.08	18.11	15.82	18.48
b* AVERAGE	8.18	9.41	7.55	7.96	7.49	8.12
Date Collected	3/16/12		Day	8		
POS Building	Cooler A		Cooler B		Cooler C	
Sample Number	Control 24	Test 5 #3	Control 4	Test 0 #3	Control 44	Test UV #3
Cooler Observations	Package not sealed due to food particles.					
R&D Lab	Control 24	Test 5 #3	Control 4	Test 0 #3	Control 44	Test UV #3
MOCON - CO2 (%)	19.7	1.1	20.1	18.1	20.3	18.7
MOCON - O2 (%)	0.10	15.00	0.16	1.21	0.04	0.09
	Control 24	Test 5 #3	Control 4	Test 0 #3	Control 44	Test UV #3
L* (1)	60.51	59.65	61.81	61.89	60.22	60.24
a* (1)	18.33	14.18	16.88	16.63	18.67	17.55
b* (1)	8.27	8.22	8.18	9.34	8.45	8.93
L* (2)	59.92	60.23	61.74	62.44	59.18	59.83
a* (2)	18.61	13.63	16.99	16.25	19.33	17.72
b* (2)	8.47	8.69	8.94	9.77	9.10	8.04
L* (3)	60.97	61.31	61.65	62.08	60.45	58.81
a* (3)	17.66	12.80	16.68	16.03	17.44	18.51
b* (3)	8.72	8.89	8.91	10.12	8.76	9.00
L* AVERAGE	60.47	60.40	61.73	62.14	59.95	59.63
a* AVERAGE	18.20	13.54	16.85	16.30	18.48	17.93
b* AVERAGE	8.49	8.60	8.68	9.74	8.77	8.66

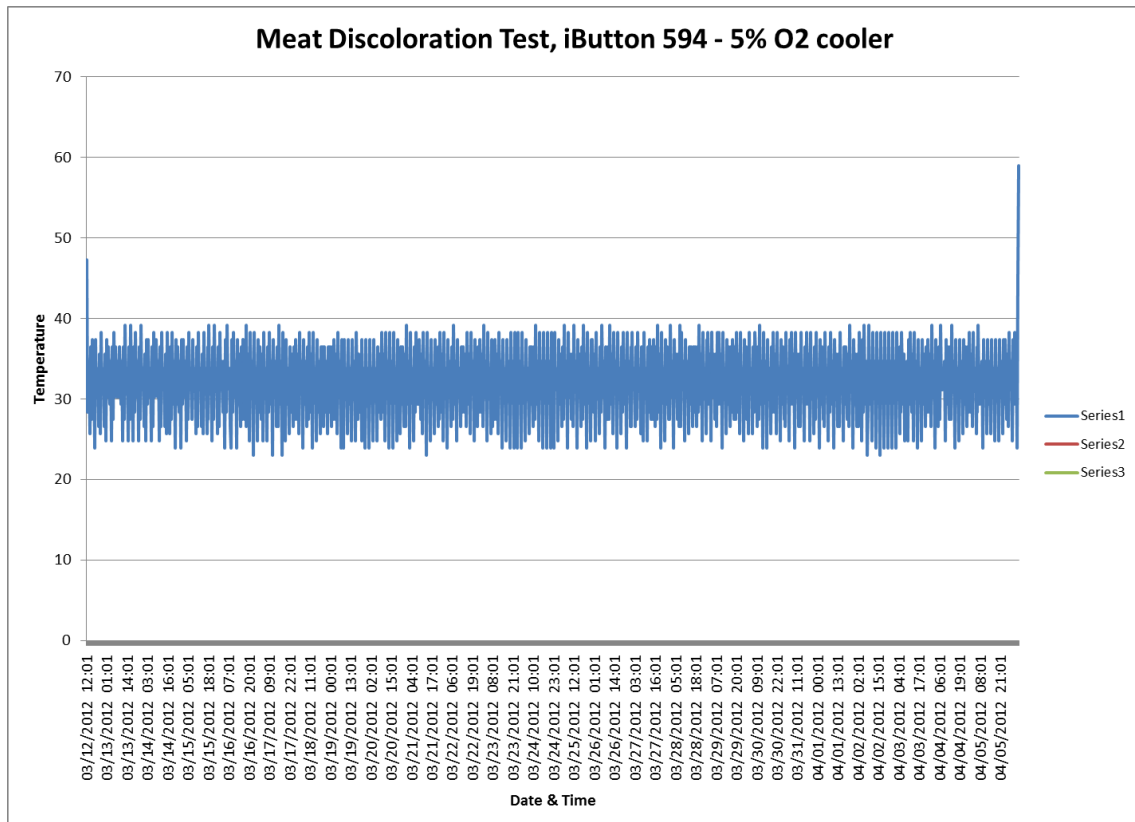
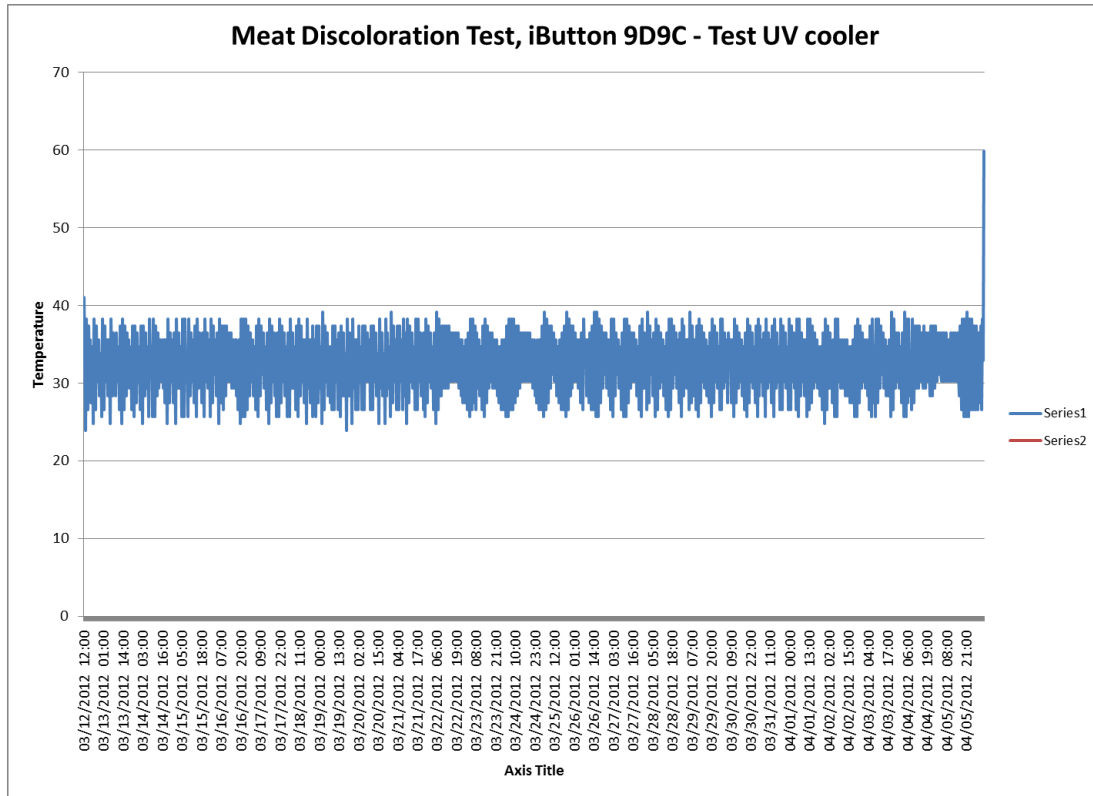


<b>WEEK 2 March 19-23, 2012</b>						
Date Collected	3/19/12		Day	11		
OS Building	Cooler A		Cooler B		Cooler C	
Sample Name	Control 23	Test 5 #14	Control 3	Test 0 #14	Control 43	Test UV #14
Cooler Observations						
R&D Lab	Control 23	Test 5 #14	Control 3	Test 0 #14	Control 43	Test UV #14
MOCON -	20.1	8.1	20.7	18.9	19.9	18.2
MOCON -	0.06	8.38	0.02	0.65	0.05	0.00
	Control 23	Test 5 #14	Control 3	Test 0 #14	Control 43	Test UV #14
L* (1)	59.34	57.22	57.99	64.47	62.62	57.01
a* (1)	18.07	12.37	19.01	10.34	16.64	17.26
b* (1)	8.40	9.44	6.86	8.28	7.38	7.39
L* (2)	60.35	56.24	58.97	63.17	62.68	57.65
a* (2)	17.62	12.88	18.28	10.26	16.33	16.58
b* (2)	8.05	9.65	7.23	8.97	7.43	6.78
L* (3)	60.26	56.28	59.32	60.69	61.29	59.56
a* (3)	17.49	12.99	18.01	10.74	16.77	15.86
b* (3)	7.74	9.04	6.42	7.79	7.82	6.52
L* AVERAGE	59.98	56.58	58.76	62.78	62.20	58.07
a* AVERAGE	17.73	12.75	18.43	10.45	16.58	16.57
b* AVERAGE	8.06	9.38	6.84	8.35	7.54	6.90
Date Collected	3/21/12		Day	13		
OS Building	Cooler A		Cooler B		Cooler C	
Sample Name	Control 34	Test 5 #13	Control 14	Test 0 #13	Control 54	Test UV #13
Cooler Observations	Gray					
R&D Lab	Control 34	Test 5 #13	Control 14	Test 0 #13	Control 54	Test UV #13
MOCON -	20.7	10.3	20.6	17.0	20.1	19.8
MOCON -	0.012	6.92	0.002	1.91	0.0004	0.002
	Control 34	Test 5 #13	Control 14	Test 0 #13	Control 54	Test UV #13
L* (1)	61.45	57.71	61.60	58.02	61.43	65.87
a* (1)	15.68	11.39	17.66	14.53	14.43	11.54
ΔE* (1)	0.72	6.76	1.31	4.05	0.33	5.41
L* (2)	61.62	57.79	60.68	57.26	61.08	64.04
a* (2)	16.23	11.48	17.62	14.26	14.49	13.34
ΔE* (2)	0.44	6.00	0.92	4.72	0.50	2.84
L* (3)	60.59	58.44	60.74	58.19	65.87	60.49
a* (3)	17.01	11.37	17.45	14.77	11.54	14.59
ΔE* (3)	1.40	5.76	0.81	3.75	5.41	1.08
L* AVERAGE	61.22	57.98	61.01	57.82	62.79	63.47
a* AVERAGE	16.31	11.41	17.58	14.52	13.49	13.16
Date Collected	3/23/12		Day	15		
OS Building	Cooler A		Cooler B		Cooler C	
Sample Name	Control 33	Test 5 #12	Control 13	Test 0 #12	Control 53	Test UV #12
Cooler Observations						
R&D Lab	Control 33	Test 5 #12	Control 13	Test 0 #12	Control 53	Test UV #12
MOCON -	20.3	15.7	20.1	18.0	20.4	19.2
MOCON -	0.052	2.57	0.003	1.38	0.007	0.205
	Control 33	Test 5 #12	Control 13	Test 0 #12	Control 53	Test UV #12
L* (1)	56.73	59.06	60.44	60.97	58.62	57.36
a* (1)	18.08	14.94	17.15	13.75	17.49	16.24
ΔE* (1)	3.72	1.63	2.18	5.08	3.18	4.32
L* (2)	59.04	59.37	58.48	63.06	61.84	57.03
a* (2)	16.39	14.85	18.06	13.98	15.91	16.63
ΔE* (2)	1.01	1.59	0.21	6.26	0.80	5.39
L* (3)	60.28	59.61	58.19	63.72	62.53	55.57
a* (3)	15.83	15.01	18.36	14.17	14.94	16.91
b* (3)	7.43	7.85	8.13	8.84	6.74	9.01
ΔL* (3)	0.34	-0.32	-0.35	5.19	1.06	-5.91
Δa* (3)	-0.37	-1.19	0.15	-4.05	-1.39	0.58
Δb* (3)	-0.75	-0.33	0.30	1.00	0.20	2.48
ΔE* (3)	0.91	1.28	0.48	6.65	1.76	6.43
L* AVERAGE	58.68	59.35	59.04	62.58	61.00	56.65
a* AVERAGE	16.77	14.93	17.86	13.97	16.11	16.59

<b>WEEK 3</b> March 26-30, 2012						
Date Collected	3/26/12		Day	18		
OS Building	Cooler A		Cooler B		Cooler C	
Sample Name	Control 32	Test 5 #11	Control 12	Test 0 #11	Control 52	Test UV #1
Cooler Observations						
R&D Lab	Control 32	Test 5 #11	Control 12	Test 0 #11	Control 52	Test UV #1
MOCON -	20.1	15.3	19.8	18.7	19.8	18.6
MOCON -	0.017	3.00	0.008	0.001	0.003	0.32
	Control 32	Test 5 #11	Control 12	Test 0 #11	Control 52	Test UV #1
L* (1)	65.83	60.27	60.69	60.09	63.00	60.61
a* (1)	14.16	12.19	16.97	17.68	16.09	15.35
b* (1)	7.53	10.41	9.13	8.58	10.37	10.51
L* (2)	65.85	62.40	61.14	59.99	63.07	60.04
a* (2)	14.08	11.96	16.25	17.79	16.00	15.77
b* (2)	7.85	9.76	8.72	9.03	10.74	9.89
L* (3)	63.91	62.11	60.87	60.31	62.68	60.98
a* (3)	14.95	11.68	16.56	17.31	15.71	14.96
b* (3)	8.65	10.84	8.75	9.44	10.37	10.05
L* AVERAGE	65.20	61.59	60.90	60.13	62.92	60.54
a* AVERAGE	14.40	11.94	16.59	17.59	15.93	15.36
b* AVERAGE	8.01	10.34	8.87	9.02	10.49	10.15
Date Collected	3/28/12		Day	20		
OS Building	Cooler A		Cooler B		Cooler C	
Sample Name	Control 31	Test 5 #10	Control 11	Test 0 #10	Control 51	Test UV #10
Cooler Observations						
R&D Lab	Control 31	Test 5 #10	Control 11	Test 0 #10	Control 51	Test UV #10
MOCON -	20.6	12.9	20.4	18.4	19.8	20.0
MOCON -	0.078	4.95	0.011	0.002	0.015	0.018
	Control 31	Test 5 #10	Control 11	Test 0 #10	Control 51	Test UV #10
L* (1)	60.76	59.82	58.83	56.62	60.44	58.53
a* (1)	16.74	13.35	17.50	18.99	17.55	17.95
ΔE* (1)	0.69	4.00	0.77	2.55	1.23	3.20
L* (2)	61.44	59.83	58.20	57.37	61.41	58.70
a* (2)	16.83	13.45	18.41	18.55	17.69	17.76
ΔE* (2)	0.21	3.86	0.83	1.76	0.07	3.02
L* (3)	60.74	59.77	56.98	58.90	61.98	58.80
a* (3)	17.36	13.24	19.04	17.60	17.27	17.45
ΔE* (3)	0.80	4.12	2.23	1.34	0.75	3.18
L* AVERAGE	60.98	59.81	58.00	57.63	61.28	58.68
a* AVERAGE	16.98	13.35	18.32	18.38	17.50	17.72
Date Collected	3/30/12		Day	22		
OS Building	Cooler A		Cooler B		Cooler C	
Sample Name	Control 30	Test 5 #20	Control 10	Test 0 #20	Control 50	Test UV #20
Cooler Observations						
R&D Lab	Control 30	Test 5 #20	Control 10	Test 0 #20	Control 50	Test UV #20
MOCON -	20.0	14.7	20.1	17.0	19.2	19.1
MOCON -	0.063	3.37	0.035	1.37	0.051	0.000
	Control 30	Test 5 #20	Control 10	Test 0 #20	Control 50	Test UV #20
L* (1)	56.88	58.36	56.80	60.43	55.48	62.76
a* (1)	19.30	9.01	19.72	9.14	19.21	13.28
ΔE* (1)	1.58	11.43	0.98	10.28	0.06	9.43
L* (2)	56.24	56.40	57.27	60.45	55.31	60.76
a* (2)	20.20	11.03	19.23	11.07	19.22	15.81
ΔE* (2)	0.15	9.18	0.31	8.50	0.15	6.32
L* (3)	55.28	55.46	58.08	59.05	55.11	60.70
a* (3)	20.30	11.26	18.61	13.32	19.22	15.41
b* (3)	8.94	10.02	8.70	9.73	9.17	9.53
ΔL* (3)	-0.97	-0.80	0.72	1.69	-0.34	5.24
Δa* (3)	0.18	-8.86	-0.33	-5.62	-0.02	-3.84
Δb* (3)	-0.07	1.01	-0.17	0.85	0.32	0.68
ΔE* (3)	0.99	8.95	0.81	5.93	0.47	6.54
L* AVERAGE	56.13	56.74	57.38	59.98	55.30	61.41
a* AVERAGE	19.93	10.43	19.19	11.18	19.22	14.83

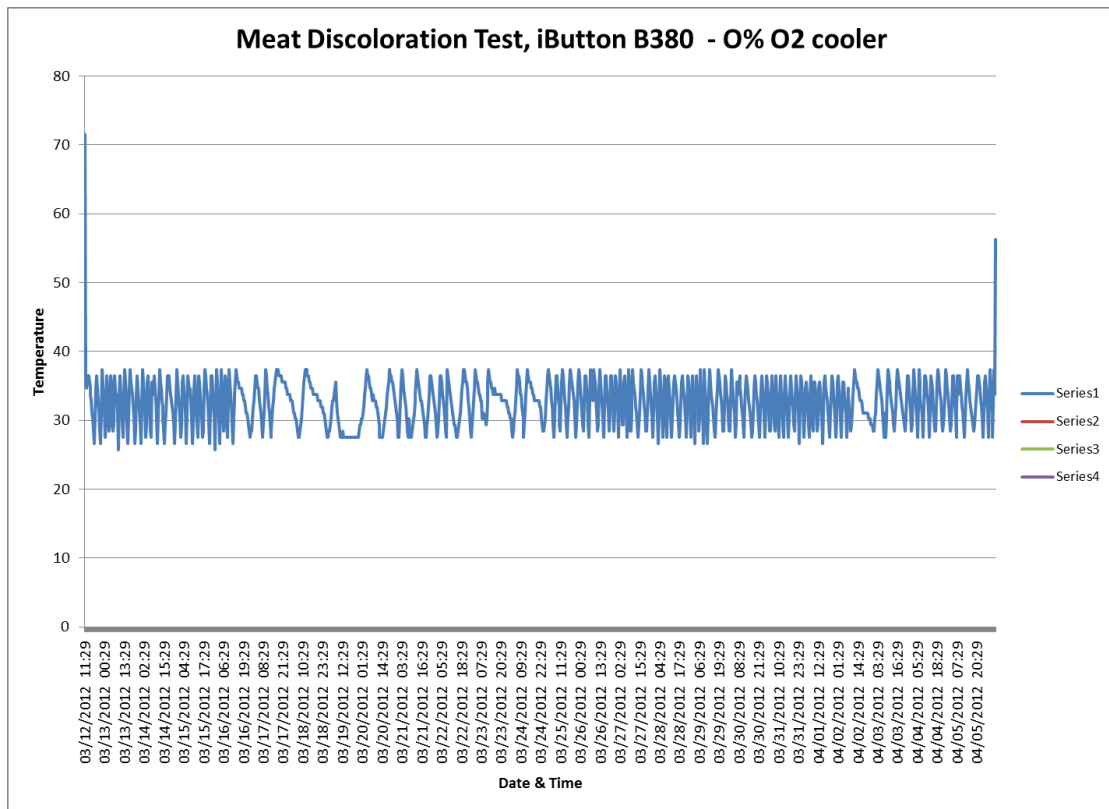
<b>WEEK 4</b>	<b>March 31, 2012</b>				
<b>Date Collected</b>	4/2/12		<b>Day</b>	25	
<b>OS Building</b>	<b>Cooler A</b>		<b>Cooler B</b>		<b>Cooler C</b>
<b>Sample Number</b>	<b>Control 39</b>	<b>Test 5 #19</b>	<b>Control 19</b>	<b>Test 0 #19</b>	<b>Control 59</b>
<b>Cooler Observations</b>					<b>Test UV #1</b>
<b>R&amp;D Lab</b>	<b>Control 39</b>	<b>Test 5 #19</b>	<b>Control 19</b>	<b>Test 0 #19</b>	<b>Control 59</b>
MOCON -	19.6	12.5	19.8	19.2	19.7
MOCON -	0.011	4.53	0.002	0.000	0.000
	<b>Control 39</b>	<b>Test 5 #19</b>	<b>Control 19</b>	<b>Test 0 #19</b>	<b>Control 59</b>
L* (1)	60.51	64.17	60.82	58.71	57.98
a* (1)	16.33	9.89	16.55	19.06	19.25
b* (1)	9.07	10.85	8.41	9.47	9.79
L* (2)	60.46	64.06	61.44	59.14	59.24
a* (2)	16.10	9.88	16.38	18.81	17.56
b* (2)	9.48	10.84	8.08	9.15	8.93
L* (3)	60.71	62.29	61.48	58.22	61.04
a* (3)	16.00	11.68	16.63	18.79	16.03
b* (3)	9.44	10.83	8.35	9.04	8.57
<b>L* AVERAGE</b>	60.56	63.51	61.25	58.69	59.42
<b>a* AVERAGE</b>	16.14	10.48	16.52	18.89	17.61
<b>b* AVERAGE</b>	9.33	10.84	8.28	9.22	9.10
<b>Date Collected</b>	4/4/12		<b>Day</b>	27	
<b>OS Building</b>	<b>Cooler A</b>		<b>Cooler B</b>		<b>Cooler C</b>
<b>Sample Number</b>	<b>Control 38</b>	<b>Test 5 #18</b>	<b>Control 18</b>	<b>Test 0 #18</b>	<b>Control 58</b>
<b>Cooler Observations</b>					<b>Test UV #1</b>
<b>R&amp;D Lab</b>	<b>Control 38</b>	<b>Test 5 #18</b>	<b>Control 18</b>	<b>Test 0 #18</b>	<b>Control 58</b>
MOCON -	19.6	12.8	20.0	19.4	19.3
MOCON -	0.069	4.68	0.030	0.000	0.005
	<b>Control 38</b>	<b>Test 5 #18</b>	<b>Control 18</b>	<b>Test 0 #18</b>	<b>Control 58</b>
L* (1)	60.07	59.23	59.81	57.14	57.30
a* (1)	17.34	13.81	17.67	18.87	19.15
ΔE* (1)	1.44	3.61	0.71	2.80	1.82
L* (2)	60.91	58.90	59.17	57.46	58.66
a* (2)	16.62	14.29	17.81	18.87	18.48
ΔE* (2)	0.41	3.58	0.78	2.81	0.30
L* (3)	60.15	58.99	58.89	57.19	58.81
a* (3)	17.06	14.84	18.09	18.24	18.40
ΔE* (3)	1.24	2.93	1.11	2.87	0.51
<b>L* AVERAGE</b>	60.38	59.04	59.29	57.26	58.26
<b>a* AVERAGE</b>	17.01	14.31	17.86	18.66	18.68
<b>Date Collected</b>	4/6/12		<b>Day</b>	29	
<b>OS Building</b>	<b>Cooler A</b>		<b>Cooler B</b>		<b>Cooler C</b>
<b>Sample Number</b>	<b>Control 37</b>	<b>Test 5 #17</b>	<b>Control 17</b>	<b>Test 0 #17</b>	<b>Control 57</b>
<b>Cooler Observations</b>					<b>Test UV #1</b>
<b>R&amp;D Lab</b>	<b>Control 37</b>	<b>Test 5 #17</b>	<b>Control 17</b>	<b>Test 0 #17</b>	<b>Control 57</b>
MOCON -	22.9	14.3	21.9	21.6	22.2
MOCON -	0.051	5.00	0.061	0.019	0.032
	<b>Control 37</b>	<b>Test 5 #17</b>	<b>Control 17</b>	<b>Test 0 #17</b>	<b>Control 57</b>
L* (1)	58.89	57.91	58.60	60.04	60.85
a* (1)	18.85	14.41	18.05	18.71	17.24
ΔE* (1)	1.40	4.00	0.58	1.47	2.02
L* (2)	59.46	57.94	59.00	62.20	62.54
a* (2)	17.73	14.39	17.98	17.31	16.83
ΔE* (2)	0.19	4.10	0.18	3.13	0.22
L* (3)	59.42	56.83	58.43	61.49	62.17
a* (3)	17.67	13.86	18.16	17.63	16.44
b* (3)	8.71	10.43	9.33	9.05	9.12
ΔL* (3)	-0.14	-2.73	-0.70	2.35	-0.57
Δa* (3)	0.05	-3.77	0.31	-0.22	-0.38
Δb* (3)	0.13	1.84	0.53	0.25	-0.30
ΔE* (3)	0.19	5.00	0.93	2.38	0.75
<b>L* AVERAGE</b>	59.26	57.56	58.68	61.24	61.85
<b>a* AVERAGE</b>	18.08	14.22	18.06	17.88	16.84
<b>Date Collected</b>	4/6/12		<b>Day</b>	29	
<b>SANDWICHES USED FOR DAILY PHOTOS AT POINT OF SALE BUILDING</b>					
<b>OS Building</b>	<b>Cooler A</b>		<b>Cooler B</b>		<b>Cooler C</b>
<b>Sample Number</b>	<b>Control 40</b>	<b>Test 5 #7</b>	<b>Control 20</b>	<b>Test 0 #7</b>	<b>Control 60</b>
<b>Cooler Observations</b>					<b>Test UV #7</b>
<b>R&amp;D Lab</b>	<b>Control 40</b>	<b>Test 5 #7</b>	<b>Control 20</b>	<b>Test 0 #7</b>	<b>Control 60</b>
MOCON -	22.3	13.3	22.3	20.7	21.6
MOCON -	0.001	5.16	0.0002	0.004	0.0001
	<b>Control 40</b>	<b>Test 5 #7</b>	<b>Control 20</b>	<b>Test 0 #7</b>	<b>Control 60</b>
L* (1)	60.58	60.90	56.94	59.23	58.17
Δb* (1)	1.25	2.26	0.55	1.01	0.13
ΔE* (1)	2.35	8.50	1.15	3.07	0.74
L* (2)	62.18	61.52	56.55	59.57	58.49
Δb* (2)	0.32	1.89	0.52	0.40	-0.25
ΔE* (2)	0.38	7.67	0.54	3.17	0.45
L* (3)	60.90	58.71	56.33	60.30	58.63
Δb* (3)	0.06	1.37	0.53	0.00	-0.25
ΔE* (3)	1.74	7.10	0.65	4.13	0.30
<b>L* AVERAGE</b>	61.22	60.38	56.61	59.70	58.43

## B.18 Temperature tracking Test 2



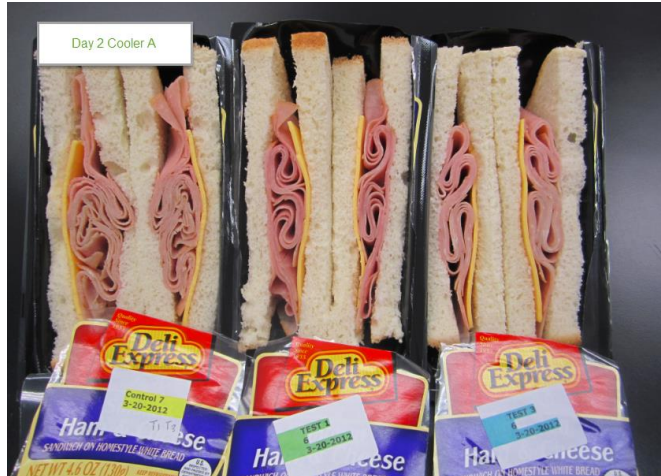


B.18 continued.



## Appendix C – Test 3 Alternate ham formulations

C.1 Visual appearance of samples from cooler A, B & C at day 2. Discoloration is only evident on the Cargill ham sample that had high oxygen. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



<b>Cooler A</b>							
Control	1	2	3	4	5	6	7
	8	9	10	11	12	13	14 pic
Test 1	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Test 3	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
<b>Cooler B</b>							
Control	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Test 6	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
<b>Cooler C</b>							
Control	36	37	38	39	40	41	42
	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Test 8	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21

C.2 Visual appearance of samples from cooler A, B & C at day 4. Some fading can be seen on the control samples when comparing the portion covered by the label to the exposed portion. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



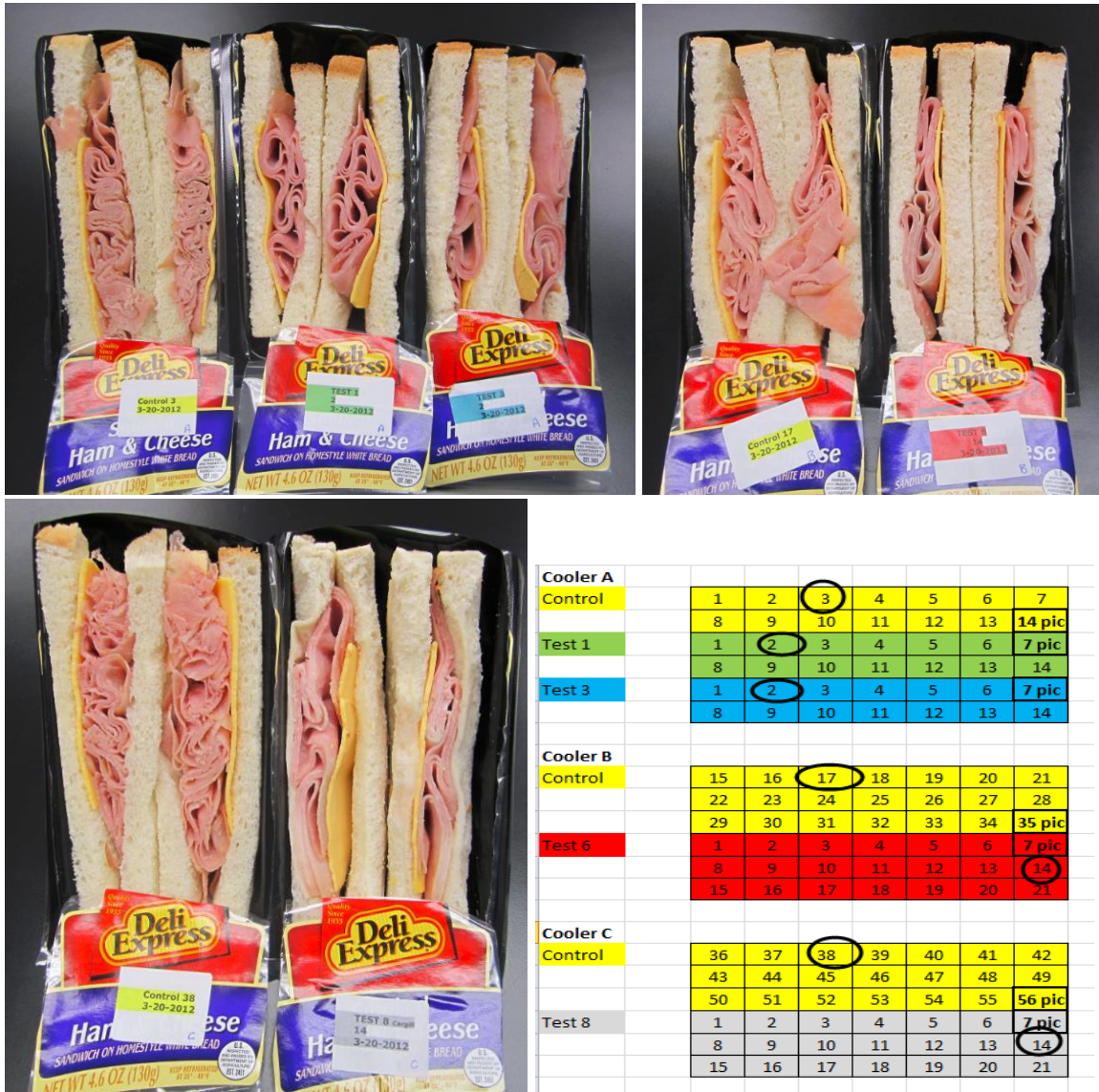
<b>Cooler A</b>							
Control	1	2	3	4	5	6	7
	8	9	10	11	12	13	14 pic
Test 1	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Test 3	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
<b>Cooler B</b>							
Control	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Test 6	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
<b>Cooler C</b>							
Control	36	37	38	39	40	41	42
	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Test 8	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21



C.3 Visual appearance of samples from cooler A, B & C at day 7. Some fading can be seen on the control samples when comparing the portion covered by the label to the exposed portion. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



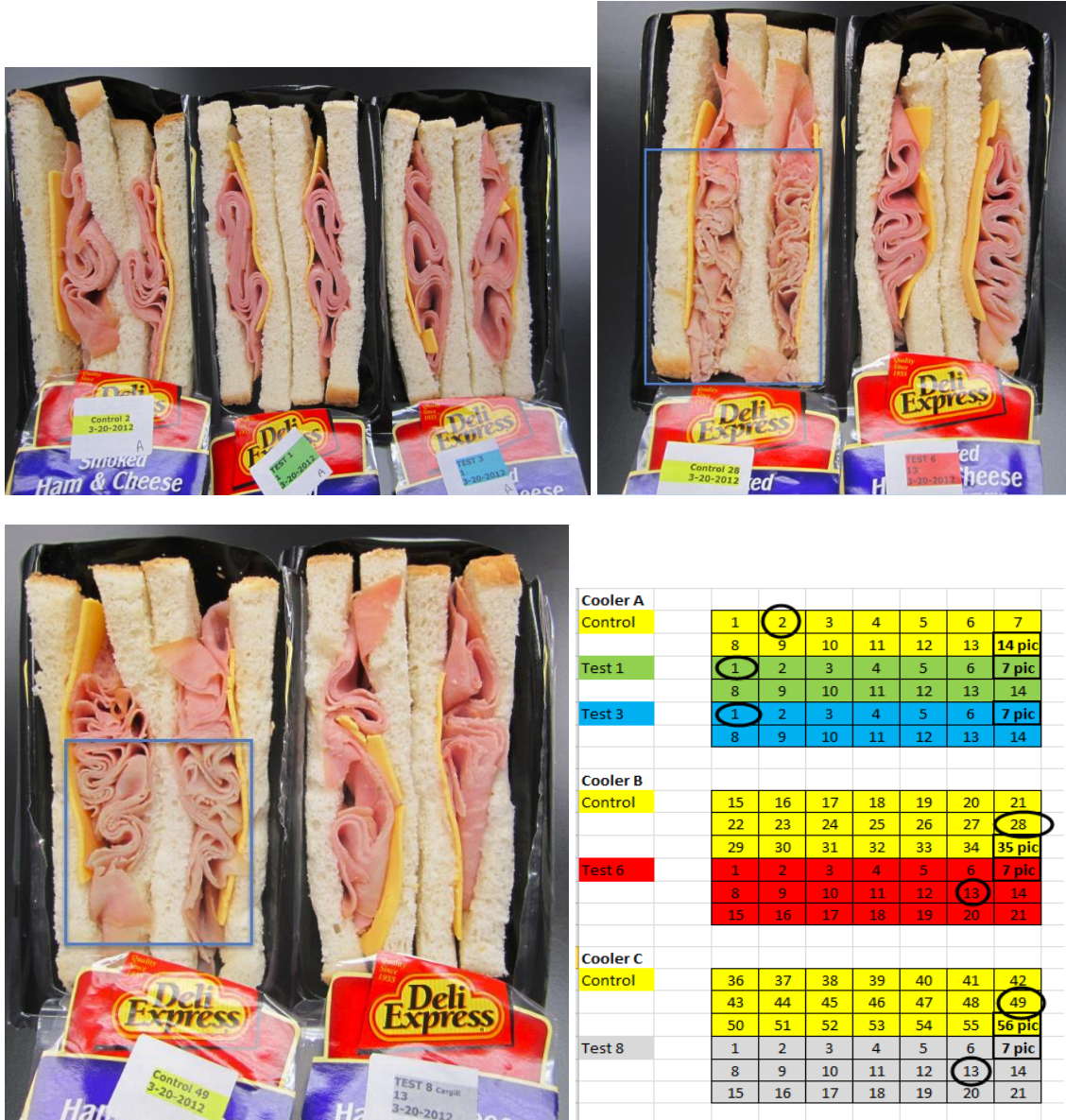
C.4 Visual appearance of samples from cooler A, B & C at day 11. Some fading can be seen on the test 6 (Rosemary) and test 8 (Cargill ham). Both samples were near the light source. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A							
Control	1	2	3	4	5	6	7
	8	9	10	11	12	13	14 pic
Test 1	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Test 3	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Cooler B							
Control	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Test 6	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
Cooler C							
Control	36	37	38	39	40	41	42
	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Test 8	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21



C.5 Visual appearance of samples from cooler A, B & C at day 14. Some fading can be seen on the control samples when comparing the portion covered by the label to the exposed portion. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



**Cooler A**

Control	1	2	3	4	5	6	7
	8	9	10	11	12	13	14 pic
Test 1	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Test 3	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14

**Cooler B**

Control	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Test 6	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21

**Cooler C**

Control	36	37	38	39	40	41	42
	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Test 8	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21

C.6 Visual appearance of samples from cooler A, B & C at day 16. Some fading can be seen on the control samples from cooler B and C as well as test 1 (current formula pilot plant) and test 3 (with fruit extract). Cooler location of the sample number is circled in the cooler layout below. All samples with discoloration on nearest the light source. The light source is on the right hand side of the cooler.



<b>Cooler A</b>							
Control	1	2	3	4	5	6	7
	8	9	10	11	12	13	14 pic
Test 1	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Test 3	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
<b>Cooler B</b>							
Control	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Test 6	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
<b>Cooler C</b>							
Control	36	37	38	39	40	41	42
	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Test 8	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21



C.7 Visual appearance of samples from cooler A, B & C at day 17.



C.8 Visual appearance of samples from cooler A, B & C at day 18.



C.9 Visual appearance of samples from cooler A, B & C at day 21.



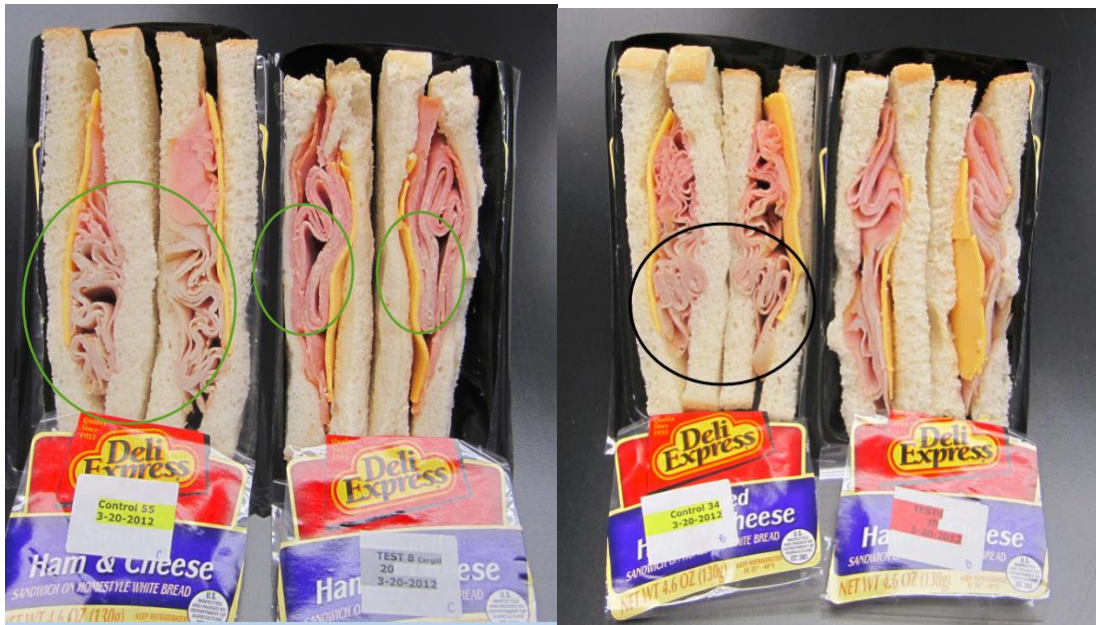


C.10 Visual appearance of samples from cooler A, B & C at day 23. Some fading can be seen on the control samples from cooler B and C as well as test 1 (current formula pilot plant) and test 3 (with fruit extract). Cooler location of the sample number is circled in the cooler layout below. All samples with discoloration on nearest the light source. The light source is on the right hand side of the cooler.



<b>Cooler A</b>							
Control	1	2	3	4	5	6	7
	8	9	10	11	12	13	14 pic
Test 1	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
Test 3	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
<b>Cooler B</b>							
Control	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31	32	33	34	35 pic
Test 6	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
<b>Cooler C</b>							
Control	36	37	38	39	40	41	42
	43	44	45	46	47	48	49
	50	51	52	53	54	55	56 pic
Test 8	1	2	3	4	5	6	7 pic
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21

C.11 Visual appearance of samples from cooler A, B & C at day 25.





C.12 Visual appearance of samples from cooler A, B & C at day 28.



C.13 Visual appearance of samples from cooler A, B & C at day 30.





C.14 Visual appearance of control sample and test 6 (with Rosemary) day 1 – 32. Both sandwiches were near the light source.

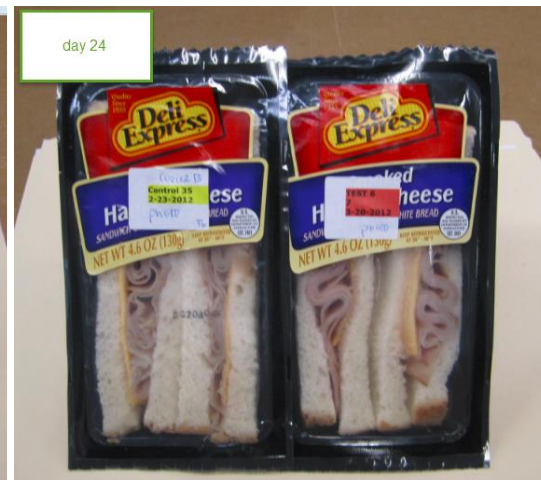
Cooler A								
Control		1	2	3	4	5	6	7
		8	9	10	11	12	13	14 pic
Test 1		1	2	3	4	5	6	7 pic
		8	9	10	11	12	13	14
Test 3		1	2	3	4	5	6	7 pic
		8	9	10	11	12	13	14
Cooler B								
Control		15	16	17	18	19	20	21
		22	23	24	25	26	27	28
		29	30	31	32	33	34	35 pic
Test 6		1	2	3	4	5	6	7 pic
		8	9	10	11	12	13	14
		15	16	17	18	19	20	21
Cooler C								
Control		36	37	38	39	40	41	42
		43	44	45	46	47	48	49
		50	51	52	53	54	55	56 pic
Test 8		1	2	3	4	5	6	7 pic
		8	9	10	11	12	13	14
		15	16	17	18	19	20	21















# C.15 Raw data for L\*a\*b\* scores and Carbon dioxide and oxygen in headspace for test 3

<b>WEEK 1 April 9-13, 2012</b>							
Date Collected	4/11/12	Day	2	Wednesday			
<i>R&amp;D Lab</i>	Control 7	Test 1 #6	Test 3 #6	Control 21	Test 6 #6	Control 42	Test 8 #6
MOCON - CO2 (%)	21.6	23.6	23.1	22.5	22.7	22.0	17.4
MOCON - O2 (%)	0.137	0.048	0.150	0.141	0.044	0.083	5.100
	Control 7	Test 1 #6	Test 3 #6	Control 21	Test 6 #6	Control 42	Test 8 #6
L* (1)	58.89	55.67	59.92	57.26	61.76	60.54	62.66
a* (1)	17.27	19.30	17.61	17.99	16.17	17.06	11.17
b* (1)	8.86	8.08	8.67	8.61	8.93	8.80	9.37
L* (2)	59.00	54.49	59.55	58.56	62.60	61.37	59.31
a* (2)	16.62	19.66	18.11	17.81	15.93	17.06	14.85
b* (2)	8.56	7.98	8.51	8.22	9.45	8.59	7.20
L* (3)	59.41	53.76	59.89	57.72	61.89	61.15	56.32
a* (3)	16.77	19.81	17.65	18.39	16.82	17.11	15.88
b* (3)	8.89	7.83	7.97	8.25	8.79	7.98	8.30
L* AVERAGE	59.10	54.64	59.79	57.85	62.08	61.02	59.43
a* AVERAGE	16.89	19.59	17.79	18.06	16.31	17.08	13.97
b* AVERAGE	8.77	7.96	8.38	8.36	9.06	8.46	8.29
Date Collected	4/13/12	Day	4	Friday			
<i>R&amp;D Lab</i>	Control 6	Test 1 #5	Test 3 #5	Control 20	Test 6 #5	Control 41	Test 8 #5
MOCON - CO2 (%)	21.7	21.5	22.1	22.0	22.4	21.8	21.4
MOCON - O2 (%)	0.167	0.073	0.112	0.155	0.096	0.084	0.144
	Control 6	Test 1 #5	Test 3 #5	Control 20	Test 6 #5	Control 41	Test 8 #5
L* (1)	61.41	55.53	59.16	58.48	66.44	59.34	59.92
a* (1)	17.04	19.26	18.63	18.35	14.24	17.44	18.12
b* (1)	7.62	7.69	9.38	8.23	8.47	8.87	8.99
L* (2)	61.50	57.54	58.02	59.30	67.60	60.10	60.88
a* (2)	17.69	17.84	19.53	18.41	13.76	17.50	18.92
b* (2)	7.33	7.69	8.03	8.69	8.58	8.25	9.34
L* (3)	61.47	57.69	57.44	58.69	67.17	59.90	61.68
a* (3)	17.49	17.68	18.69	18.93	13.34	17.30	16.85
b* (3)	8.12	7.62	7.78	8.97	8.34	8.61	7.66
L* AVERAGE	61.46	56.92	58.21	58.82	67.07	59.78	60.83
a* AVERAGE	17.41	18.26	18.95	18.56	13.78	17.41	17.96
b* AVERAGE	7.69	7.67	8.40	8.63	8.46	8.58	8.66
<b>WEEK April 16-20, 2012</b>							
Date Collected	4/16/12	Day	7	Monday			
<i>R&amp;D Lab</i>	Control 5	Test 1 #4	Test 3 #4	Control 19	Test 6 #4	Control 40	Test 8 #4
MOCON - CO2 (%)	21.5	22.7	22.2	21.8	22.4	21.8	22.2
MOCON - O2 (%)	0.534	0.119	0.298	0.114	0.142	0.168	0.101
	Control 5	Test 1 #4	Test 3 #4	Control 19	Test 6 #4	Control 40	Test 8 #4
L* (1)	56.09	61.20	58.17	57.98	62.11	57.62	55.51
a* (1)	18.96	16.75	18.46	19.85	17.09	18.82	22.46
b* (1)	8.14	8.63	8.84	9.75	8.03	8.32	8.65
L* (2)	56.11	60.61	57.62	58.71	60.61	57.23	57.71
a* (2)	19.14	16.95	19.28	19.66	18.79	19.04	20.16
b* (2)	8.70	8.36	9.33	9.09	7.85	8.67	8.71
L* (3)	56.44	61.07	57.06	59.21	61.46	58.11	62.61
a* (3)	20.17	16.80	19.48	19.14	17.76	18.83	16.52
b* (3)	9.11	8.63	9.01	8.89	8.76	9.39	7.86
L* AVERAGE	56.21	60.96	57.62	58.63	61.39	57.65	58.61
a* AVERAGE	19.42	16.83	19.07	19.55	17.88	18.90	19.71
b* AVERAGE	8.65	8.54	9.06	9.24	8.21	8.79	8.41
Date Collected	4/18/12	Day	9	Wednesday			
<i>R&amp;D Lab</i>	Control 4	Test 1 #3	Test 3 #3	Control 18	Test 6 #3	Control 39	Test 8 #3
MOCON - CO2 (%)	23.1	23.6	22.8	22.9	22.4	22.0	22.3
MOCON - O2 (%)	0.242	0.083	0.130	0.081	0.128	0.165	0.071
	Control 4	Test 1 #3	Test 3 #3	Control 18	Test 6 #3	Control 39	Test 8 #3
L* (1)	60.67	60.77	55.81	58.17	60.17	57.76	59.73
a* (1)	17.54	18.22	21.60	18.23	18.17	19.36	16.44
b* (1)	9.05	10.07	9.88	9.45	7.97	8.43	7.73
L* (2)	60.77	61.01	57.19	58.22	60.16	57.96	59.14
a* (2)	17.45	18.03	20.27	18.15	18.61	19.28	17.33
b* (2)	8.80	9.68	8.76	9.94	8.73	8.53	7.41
L* (3)	60.48	60.52	58.99	58.40	58.89	57.65	59.51
a* (3)	17.66	18.09	18.42	18.60	19.52	19.37	17.22
b* (3)	9.24	9.78	8.01	9.78	9.17	8.31	8.39
L* AVERAGE	60.64	60.77	57.33	58.26	59.74	57.79	59.46
a* AVERAGE	17.55	18.11	20.10	18.33	18.77	19.34	17.00
b* AVERAGE	9.03	9.84	8.88	9.72	8.62	8.42	7.84

Date Collected	4/20/12	Day		11 Friday			
R&D Lab	Control 3	Test 1 #2	Test 3 #2	Control 17	Test 6 #14	Control 38	Test 8 #14
MOCON - CO2 (%)	22.4	22.5	22.4	21.5	22.6	22.2	22.2
MOCON - O2 (%)	0.089	0.275	0.269	0.145	0.100	0.150	0.121
	Control 3	Test 1 #2	Test 3 #2	Control 17	Test 6 #14	Control 38	Test 8 #14
L* (1)	58.79	58.26	56.77	59.80	59.64	57.23	62.48
a* (1)	17.97	19.48	19.40	17.44	16.76	20.05	14.93
b* (1)	9.77	8.09	8.72	9.08	8.54	8.94	7.95
L* (2)	59.02	58.24	55.98	60.37	59.14	57.29	60.79
a* (2)	17.77	19.01	19.98	17.18	17.49	20.11	16.81
b* (2)	9.64	8.29	8.08	9.80	8.33	9.16	8.97
L* (3)	59.26	57.55	54.03	59.61	57.28	56.95	60.59
a* (3)	17.97	19.78	21.49	17.76	19.11	20.00	17.04
b* (3)	9.10	8.23	7.71	9.38	8.42	8.28	8.70
L* AVERAGE	59.02	58.02	55.59	59.93	58.69	57.16	61.29
a* AVERAGE	17.90	19.42	20.29	17.46	17.79	20.05	16.26
b* AVERAGE	9.50	8.20	8.17	9.42	8.43	8.79	8.54
WEEK 3 April 23-27, 2012							
Date Collected	4/23/12	Day		14			
R&D Lab	Control 2	Test 1 #1	Test 3 #1	Control 28	Test 6 #13	Control 49	Test 8 #13
MOCON - CO2 (%)	22.0	21.9	22.4	21.0	21.2	21.6	22.4
MOCON - O2 (%)	0.395	0.160	0.098	0.055	0.120	0.081	0.061
	Control 2	Test 1 #1	Test 3 #1	Control 28	Test 6 #13	Control 49	Test 8 #13
L* (1)	60.21	57.07	59.42	57.32	58.11	58.20	61.23
a* (1)	18.85	20.28	18.27	19.30	18.08	17.04	15.08
b* (1)	10.61	8.90	8.50	9.34	6.86	8.36	9.57
L* (2)	61.36	58.42	59.10	58.64	57.73	57.71	60.76
a* (2)	17.72	19.14	18.36	18.25	18.05	17.13	15.28
b* (2)	10.10	7.55	8.90	8.65	6.84	8.58	9.29
L* (3)	61.29	59.90	58.74	58.49	57.64	57.67	60.25
a* (3)	17.15	17.90	18.47	17.34	18.18	17.17	15.96
b* (3)	9.71	7.44	8.50	8.40	7.02	8.75	9.27
L* AVERAGE	60.95	58.46	59.09	58.15	57.83	57.86	60.75
a* AVERAGE	17.91	19.11	18.37	18.30	18.10	17.11	15.44
b* AVERAGE	10.14	7.96	8.63	8.80	6.91	8.56	9.38
Date Collected	4/25/12	Day		16 Wednesday			
R&D Lab	Control 1	Test 1 #14	Test 3 #14	Control 27	Test 6 #12	Control 48	Test 8 #12
MOCON - CO2 (%)	21.3	21.1	22.1	20.6	21.9	21.1	22.2
MOCON - O2 (%)	0.197	0.103	0.093	0.069	0.085	0.065	0.073
	Control 1	Test 1 #14	Test 3 #14	Control 27	Test 6 #12	Control 48	Test 8 #12
L* (1)	58.16	58.98	56.88	61.18	61.51	58.83	59.39
a* (1)	19.30	14.28	18.40	15.37	17.77	17.99	17.96
b* (1)	9.70	9.07	8.52	9.64	9.14	8.86	8.29
L* (2)	59.28	57.53	56.58	61.75	61.37	59.18	58.36
a* (2)	19.11	16.02	18.48	15.68	17.67	17.48	18.69
b* (2)	10.06	9.39	8.72	9.50	9.11	9.01	9.22
L* (3)	59.21	57.54	56.27	61.49	62.09	59.03	58.88
a* (3)	18.41	17.37	18.46	15.84	16.89	17.34	18.27
b* (3)	8.53	8.20	9.41	9.36	8.95	9.03	9.20
L* AVERAGE	58.88	58.02	56.58	61.47	61.66	59.01	58.88
a* AVERAGE	18.94	15.89	18.45	15.63	17.44	17.60	18.31
b* AVERAGE	9.43	8.89	8.88	9.50	9.07	8.97	8.90
Date Collected	4/27/12	Day		18 Friday			
R&D Lab	Control 13	Test 1 #13	Test 3 #13	Control 26	Test 6 #6	Control 47	Test 8 #11
MOCON - CO2 (%)	21.5	22.2	22.9	21.2	22.4	21.7	22.3
MOCON - O2 (%)	0.197	0.070	0.027	0.106	0.116	0.070	0.081
	Control 13	Test 1 #13	Test 3 #13	Control 26	Test 6 #6	Control 47	Test 8 #11
L* (1)	58.34	58.71	59.81	58.90	63.54	57.38	59.95
a* (1)	17.00	17.81	17.35	18.21	15.12	18.74	17.74
b* (1)	6.69	9.13	9.07	8.97	8.79	8.33	9.52
L* (2)	59.10	58.73	60.15	58.71	63.82	57.73	60.18
a* (2)	17.24	17.86	17.56	19.00	15.49	18.86	17.98
b* (2)	9.25	8.42	9.03	9.11	8.42	8.04	9.51
L* (3)	59.48	58.32	62.03	58.45	64.03	57.32	61.41
a* (3)	17.42	18.05	16.91	19.00	15.05	19.46	16.93
b* (3)	10.55	8.45	7.98	9.64	8.80	8.67	9.23
L* AVERAGE	58.97	58.59	60.66	58.69	63.80	57.48	60.51
a* AVERAGE	17.22	17.91	17.27	18.74	15.22	19.02	17.55
b* AVERAGE	8.83	8.67	8.69	9.24	8.67	8.35	9.42

WEEK 3 April 30-May 4, 2012							
Date Collected	4/30/12	Day	21				
R&D Lab	Control 12	Test 1 #12	Test 3 #12	Control 25	Test 6 #10	Control 46	Test 8 #10
MOCON - CO2 (%)	20.1	21.5	21.1	20.6	22.3	20.7	21.2
MOCON - O2 (%)	0.265	0.028	0.007	0.031	0.092	0.092	0.749
	Control 12	Test 1 #12	Test 3 #12	Control 25	Test 6 #10	Control 46	Test 8 #10
L* (1)	61.55	57.49	56.75	58.64	60.68	56.55	61.54
a* (1)	16.27	19.95	18.15	16.51	18.58	19.07	17.06
b* (1)	9.48	8.75	7.87	9.14	8.49	9.42	8.65
L* (2)	63.25	57.69	57.49	58.88	60.16	57.25	62.26
a* (2)	15.59	19.97	18.50	17.56	19.17	18.47	16.55
b* (2)	9.31	8.88	9.45	9.72	8.85	9.96	8.69
L* (3)	61.48	59.29	57.95	57.94	59.43	57.82	61.87
a* (3)	16.62	18.06	18.62	18.35	20.03	18.21	17.08
b* (3)	8.52	8.35	9.57	10.55	8.90	9.42	8.62
L* AVERAGE	62.09	58.16	57.40	58.49	60.09	57.21	61.89
a* AVERAGE	16.16	19.33	18.42	17.47	19.26	18.58	16.90
b* AVERAGE	9.10	8.66	8.96	9.80	8.75	9.60	8.65
Date Collected	5/2/12	Day	23				
R&D Lab	Control 11	Test 1 #11	Test 3 #11	Control 24	Test 6 #21	Control 45	Test 8 #21
MOCON - CO2 (%)	20.5	22.0	22.3	20.1	21.8	19.9	21.1
MOCON - O2 (%)	0.108	0.036	0.002	0.024	0.000	0.022	0.000
	Control 11	Test 1 #11	Test 3 #11	Control 24	Test 6 #21	Control 45	Test 8 #21
L* (1)	53.93	60.08	56.90	59.35	59.81	58.16	58.93
a* (1)	20.21	18.60	19.98	18.50	18.22	18.29	16.66
b* (1)	8.10	9.84	8.35	9.40	9.05	7.90	7.67
L* (2)	55.12	60.82	57.13	59.13	59.36	58.22	59.76
a* (2)	19.50	18.00	19.55	18.21	18.30	18.56	16.17
b* (2)	8.03	9.41	8.22	9.20	8.58	7.69	7.39
L* (3)	55.46	60.26	56.31	58.81	58.81	58.08	59.51
a* (3)	19.47	17.77	19.83	18.78	17.74	18.63	16.29
b* (3)	7.60	8.66	8.48	9.26	8.44	8.36	7.46
L* AVERAGE	54.84	60.39	56.78	59.10	59.33	58.15	59.40
a* AVERAGE	19.73	18.12	19.79	18.50	18.09	18.49	16.37
b* AVERAGE	7.91	9.30	8.35	9.29	8.69	7.98	7.51
Date Collected	5/4/12	Day	25				
R&D Lab	Control 10	Test 1 #10	Test 3 #10	Control 34	Test 6 #20	Control 55	Test 8 #20
MOCON - CO2 (%)	21.4	20.5	21.3	20.1	22.1	20.4	22.0
MOCON - O2 (%)	0.093	0.021	0.001	0.000	0.006	0.070	0.002
	Control 10	Test 1 #10	Test 3 #10	Control 34	Test 6 #20	Control 55	Test 8 #20
L* (1)	61.05	57.93	55.99	59.45	62.84	63.92	58.66
a* (1)	17.17	18.79	19.23	17.52	15.61	13.45	18.63
b* (1)	9.22	8.48	8.51	10.53	9.68	8.60	9.56
L* (2)	60.54	58.21	55.97	59.30	64.31	63.99	59.43
a* (2)	17.84	18.74	19.83	17.10	15.40	13.00	18.06
b* (2)	9.43	8.93	8.96	9.94	9.57	8.37	9.11
L* (3)	60.46	58.12	55.59	59.70	63.97	63.41	59.74
a* (3)	18.14	18.61	19.87	16.47	15.91	12.88	17.62
b* (3)	8.86	9.23	8.91	9.25	9.68	8.53	8.89
L* AVERAGE	60.68	58.09	55.85	59.48	63.71	63.77	59.28
a* AVERAGE	17.72	18.71	19.64	17.03	15.64	13.11	18.10
b* AVERAGE	9.17	8.88	8.79	9.91	9.64	8.50	9.19
WEEK 3 April 30-May 4, 2012							
Date Collected	5/7/12	Day	28				
R&D Lab	Control 9	Test 1 #9	Test 3 #9	Control 33	Test 6 #19	Control 54	Test 8 #19
MOCON - CO2 (%)	0.1	22.5	21.3	21.4	22.5	20.9	21.9
MOCON - O2 (%)	20.600	0.098	0.034	0.007	0.002	0.051	0.001
	Control 9	Test 1 #9	Test 3 #9	Control 33	Test 6 #19	Control 54	Test 8 #19
L* (1)	55.17	54.18	55.39	56.44	59.64	57.56	58.34
a* (1)	12.37	19.87	21.01	20.31	17.96	18.49	16.89
b* (1)	9.23	8.37	8.76	9.05	8.05	8.64	8.38
L* (2)	57.51	53.39	54.83	56.62	60.40	57.68	60.08
a* (2)	10.92	20.20	21.52	20.09	17.68	18.35	16.23
b* (2)	10.62	7.01	8.85	8.91	8.27	8.47	7.63
L* (3)	57.33	52.50	55.05	56.65	58.81	58.00	61.04
a* (3)	11.36	21.15	21.35	19.98	18.69	18.60	15.54
b* (3)	11.16	7.92	8.97	8.93	8.45	8.78	7.95
L* AVERAGE	56.67	53.36	55.09	56.57	59.62	57.75	59.82
a* AVERAGE	11.55	20.41	21.29	20.13	18.11	18.48	16.22
b* AVERAGE	10.34	7.77	8.86	8.96	8.26	8.63	7.99

Date Collected	5/9/12		Day	30			
<i>R&amp;D Lab</i>	Control 8	Test 1 #8	Test 3 #8	Control 32	Test 6 #18	Control 53	Test 8 #18
MOCON - CO2 (%)	21.6	21.5	22.9	20.5	21.1	20.1	21.4
MOCON - O2 (%)	0.13	0.07	0.00	0.04	0.04	0.01	0.00
	Control 8	Test 1 #8	Test 3 #8	Control 32	Test 6 #18	Control 53	Test 8 #18
L* (1)	58.07	58.06	56.71	56.15	59.36	58.88	62.13
a* (1)	18.60	19.16	19.03	19.95	18.97	18.03	16.14
b* (1)	9.73	9.21	8.63	8.58	8.55	9.19	8.39
L* (2)	57.89	59.59	57.40	56.06	59.11	58.62	61.46
a* (2)	18.61	18.22	18.92	20.00	19.49	18.73	16.36
b* (2)	9.32	8.84	8.96	8.24	8.69	9.39	8.10
L* (3)	58.38	59.56	56.77	56.67	59.04	58.62	61.60
a* (3)	18.38	17.87	18.95	19.55	19.27	18.54	16.14
b* (3)	8.87	8.43	9.47	8.66	8.69	8.92	8.04
L* AVERAGE	58.11	59.07	56.96	56.29	59.17	58.71	61.73
a* AVERAGE	18.53	18.42	18.97	19.83	19.24	18.43	16.21
b* AVERAGE	9.31	8.83	9.02	8.49	8.64	9.17	8.18
Date Collected	5/9/12		Day	Samples photographed daily.			
<i>R&amp;D Lab</i>	Control 14	Test 1 #7	Test 3 #7	Control 35	Test 6 #7	Control 56	Test 8 #7
MOCON - CO2 (%)	21.9	21.4	22.9	20.0	21.2	19.8	21.4
MOCON - O2 (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Control 14	Test 1 #7	Test 3 #7	Control 35	Test 6 #7	Control 56	Test 8 #7
L* (1)	56.19	57.42	57.60	58.70	60.71	57.07	62.25
a* (1)	18.95	18.68	18.34	16.74	16.46	16.41	15.02
b* (1)	8.71	7.96	8.16	9.24	8.36	7.15	8.43
L* (2)	56.88	57.45	58.34	59.00	60.56	56.41	61.95
a* (2)	18.58	18.78	18.71	15.95	16.64	16.52	15.42
b* (2)	8.12	7.61	8.65	8.68	9.03	8.18	8.69
L* (3)	56.49	56.90	58.09	58.23	57.38	56.96	61.29
a* (3)	18.54	19.17	18.44	15.77	18.77	16.47	16.08
b* (3)	8.09	8.07	8.41	8.26	9.59	8.07	8.59
L* AVERAGE	56.52	57.26	58.01	58.64	59.55	56.81	61.83
a* AVERAGE	18.69	18.88	18.50	16.15	17.29	16.47	15.51
b* AVERAGE	8.31	7.88	8.41	8.73	8.99	7.80	8.57



## Product Specification

**OXY'LESS® CS**

**Ref :  
BA050624**

*This specification sheet cancels and replaces all previous publications : March 23, 2010*

**\* Description :**

Extract obtained from rosemary leaves.  
Botanical name : *Rosmarinus officinalis* L.

**\* Composition :**

Natural extract , Arabic gum

**\* Regulations status :**

This rosemary extract complies with the definition of natural flavor according to US Code of Federal Regulation 21CFR101.22.

**\* Specifications :**

**Sensory quality :**

Aspect :	Powder
Color :	Clear beige
Flavor :	Characteristic
Solubility :	Oil dispersible

**Analytical quality :**

Carosolic acid content :	> 20 %
Protection factor :	> 12*
Dry extract :	92 - 98 %

**Microbiological quality :**

Total plate count :	< 1000 cfu/g
Yeasts and molds :	< 100 cfu/g
Coliforms :	< 10 cfu/g*
E.coli :	Negative/g*
Salmonella :	Negative/25g*

*\*Control Plan, Analysis performed once a year.*

**\* Packaging :**

Polyethylene bag + cardboard box: 10 and 25 kg net

**\* Storage conditions :**

Temperature <12 °C, sheltered from light, moisture and oxygen.

**\* Shelf life :**

12 months under the previously mentioned conditions and in its original packaging.

1/1

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E-mail : [naturex@naturex.com](mailto:naturex@naturex.com) - Website : [www.naturex.com](http://www.naturex.com)

## C.17 Fruit Extract additive



### Product Specification

#### ACEROLA FRUIT 17

Ref :  
BA057013

*This specification sheet cancels and replaces all previous publications : March 17, 2011*

- **Description :**

Extract obtained from acerola fruits.  
Botanical name : *Malpighia glabra* L.  
Raw material origin : Brazil

- **Composition :**

Natural extract , Glucose syrup

- **Regulations status :**

Natural flavouring of the named source according to European Regulation 1334/2008/EC. Natural flavoring according to US Code of Federal Regulation 21CFR101.22.

- **Specifications :**

- **Sensory quality :**

Aspect :	Powder
Color :	Brown-orange
Flavor :	Characteristic
Solubility :	Water soluble

- **Analytical quality :**

Naturally occurring ascorbic acid. :	> 17 %
Particle size :	100% through 420 µm*
Water (Karl Fischer) :	< 8 %
pH (2 % in water) :	5.5 - 6.5

- **Microbiological quality :**

- **Microbiological quality :**

Total plate count :	< 1 000 cfu/g
Yeasts and molds :	< 100 cfu/g

\*Control Plan, Analysis performed once a year.

- **Packaging :**

Polyethylene bag +cardboard box: 25kg

- **Storage conditions :**

1/2

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E-mail : naturex@naturex.com - Website : www.naturex.com



### Product Specification

#### ACEROLA FRUIT 17

Ref :  
BA057013

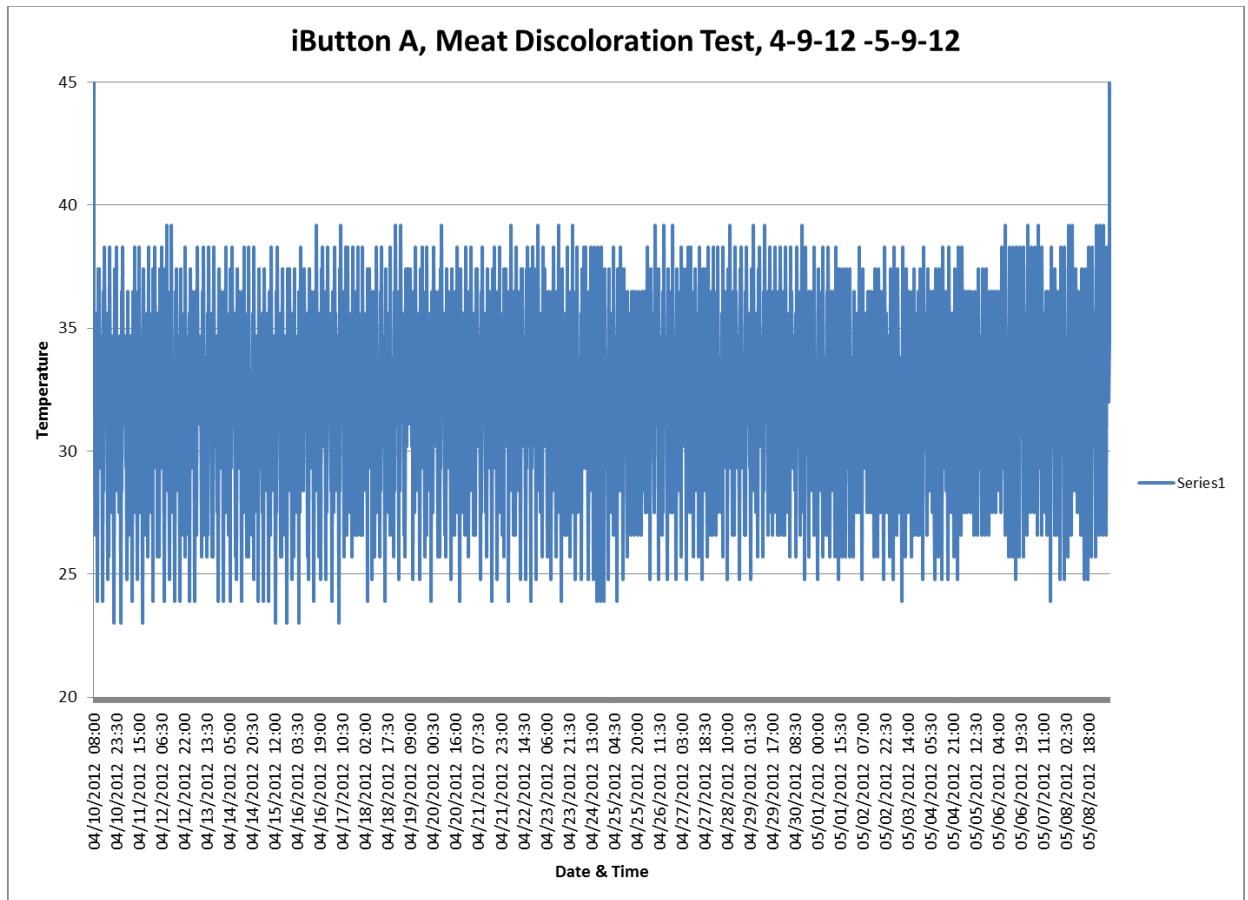
*This specification sheet cancels and replaces all previous publications : March 17, 2011*

Room temperature, sheltered from light, moisture and oxygen.

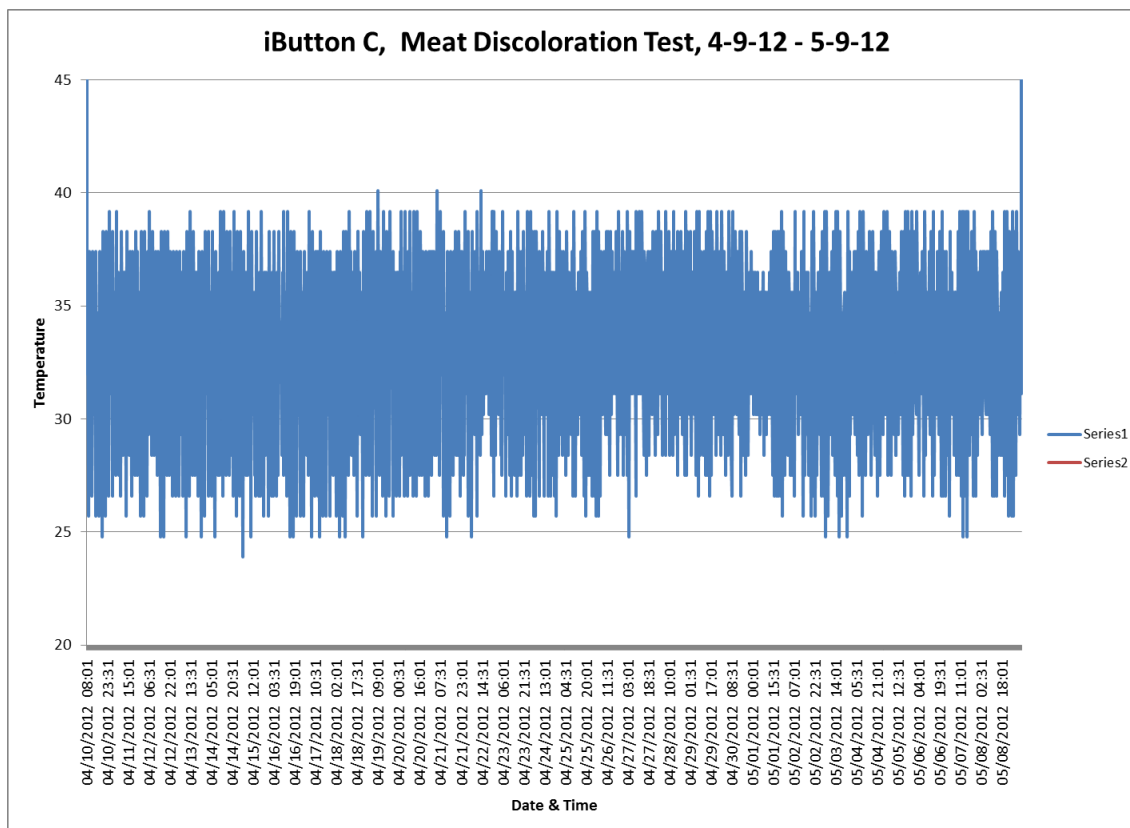
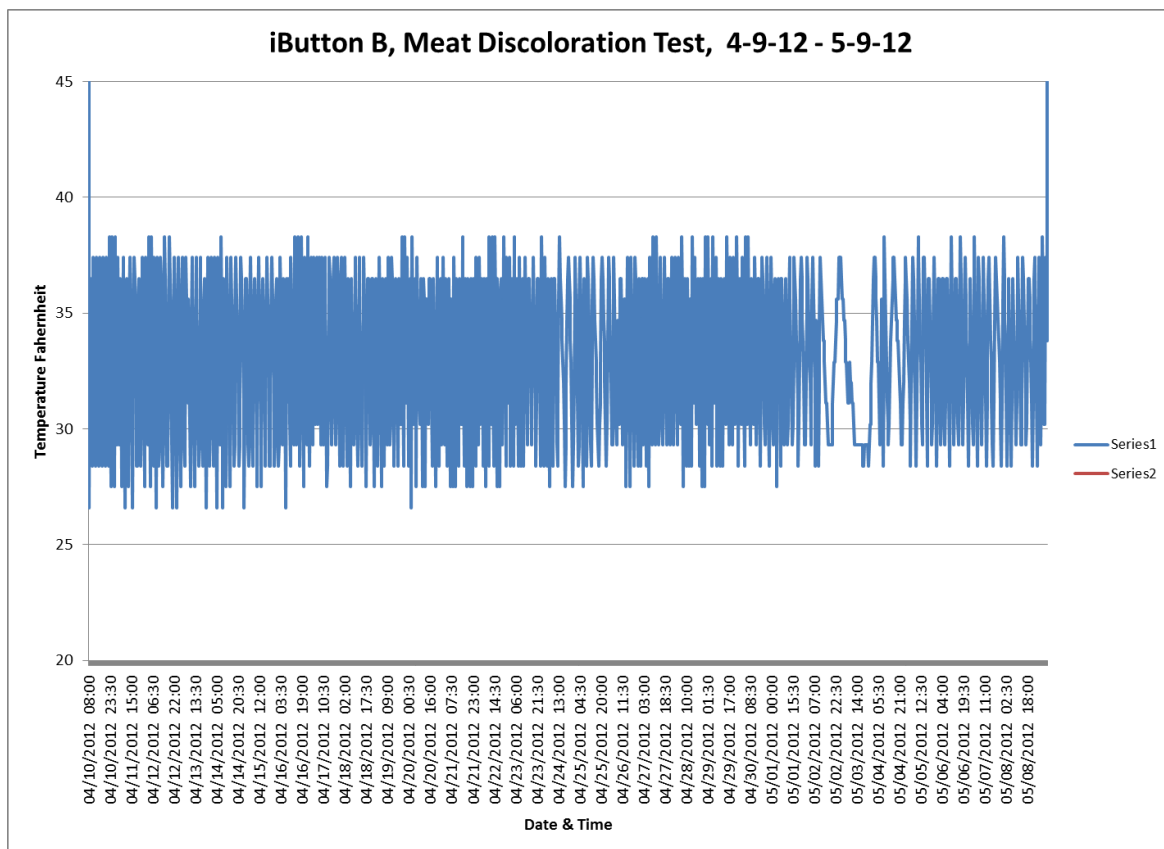
- **Shelf life :**

24 months under the previously mentioned conditions and in its original packaging.

### C.18 Temperature tracking data from test 3







## Appendix D – Test 4 Ferrous based oxygen scavenging sachet

**D.1** Visual appearance of the control samples compared to the scavenger test samples at day 3. Discoloration is only evident on the test sample that had high oxygen. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A								
Control	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
	Bottom shelf - 6	C35	C36	C37	C38	C39	C40 Pic	
Cooler B								
Test	Top - 1	1	2	3	4	5	6	7
		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
		21	22	23	24	25	26	27
		28	29	30	31	32	33	34
	Bottom shelf - 6	35	36	36	38	39	40 Pic	

**D.2** Visual appearance of the control samples compared to the scavenger test samples at day 5. No perceptible difference in color across all samples. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



<b>Cooler A</b>								
Control	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
	Bottom shelf - 6	C35	C36	C37	C38	C39	C40 Pic	
<b>Cooler B</b>								
Test	Top - 1	1	2	3	4	5	6	7
		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
		21	22	23	24	25	26	27
		28	29	30	31	32	33	34
	Bottom shelf - 6	35	36	36	38	39	40 Pic	

**D.3** Visual appearance of the control samples compared to the scavenger test samples at day 7. Discoloration is only evident on the test sample that had high oxygen. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



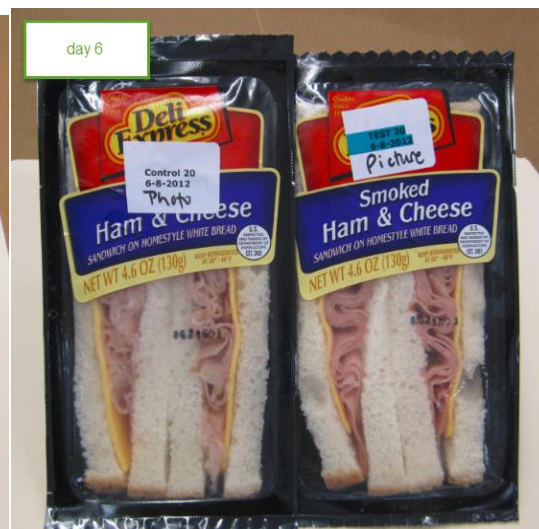
<b>Cooler A</b>								
Control	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
	Bottom shelf - 6	C35	C36	C37	C38	C39	C40 Pic	
<b>Cooler B</b>								
Test	Top - 1	1	2	3	4	5	6	7
		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
		21	22	23	24	25	26	27
		28	29	30	31	32	33	34
	Bottom shelf - 6	35	36	36	38	39	40 Pic	



**D.4** Pictures of Test and control sample number 20 through day 3 – day 31. These were taken in an effort to track the color changes of the same sandwich throughout the shelf life. The disadvantage of this method is oxygen content and color score are unknowns. The color of the samples changed very little in both applications.

<b>Cooler A</b>								
Control	Top - 1	C1	C2	C3	C4	C5	C6	C7
		C8	C9	C10	C11	C12	C13	C14
		C15	C16	C17	C18	C19	C20 Pic	
		C21	C22	C23	C24	C25	C26	C27
		C28	C29	C30	C31	C32	C33	C34
	Bottom shelf - 6	C35	C36	C37	C38	C39	C40 Pic	
<b>Cooler B</b>								
Test	Top - 1	1	2	3	4	5	6	7
		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
		21	22	23	24	25	26	27
		28	29	30	31	32	33	34
	Bottom shelf - 6	35	36	37	38	39	40 Pic	





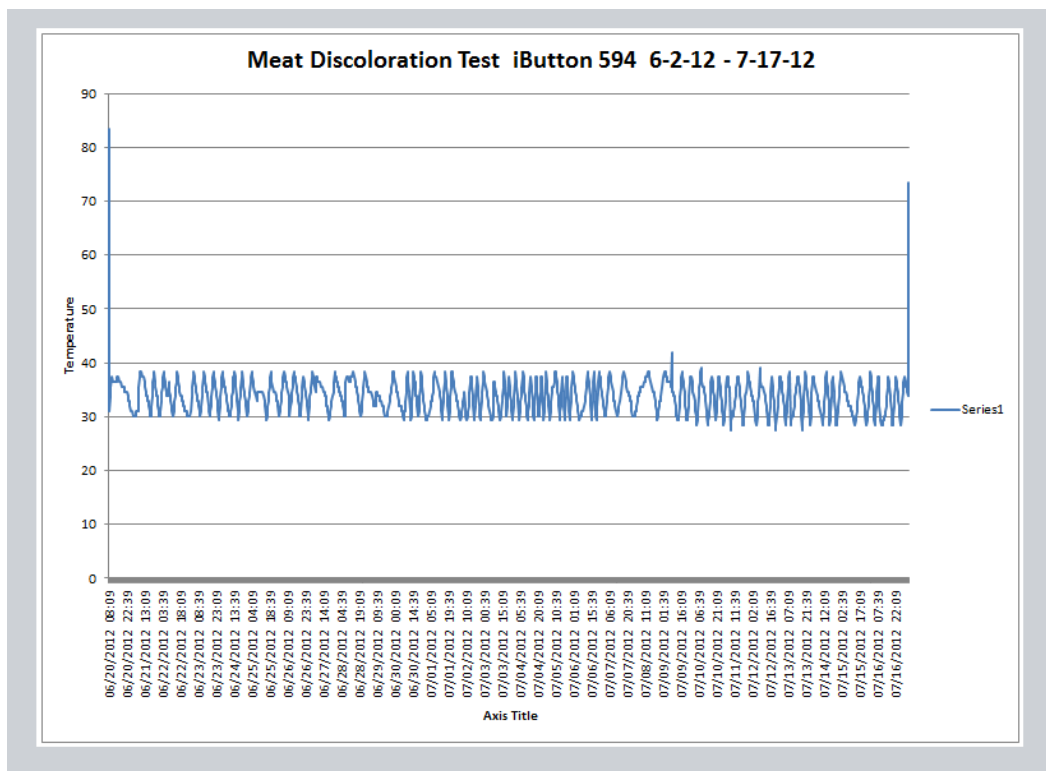
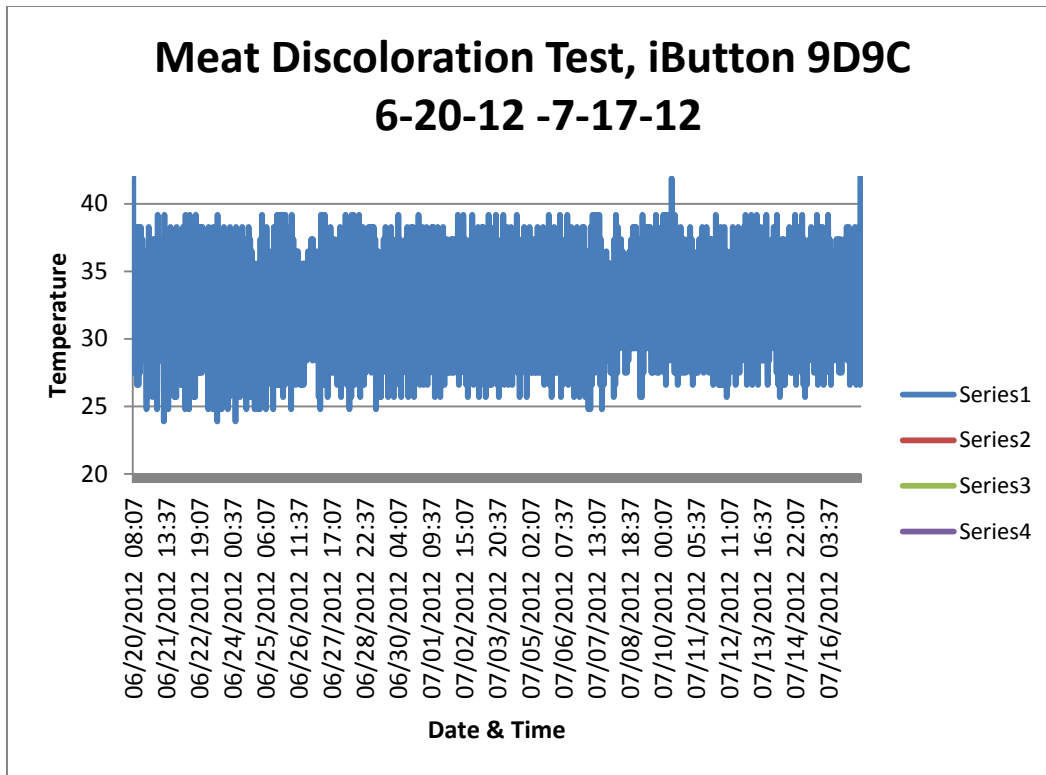








## D.5 Cooler A and B temperature tracking (in Fahrenheit) in Test 4



## D.6 Multisorb® D-30 cc sachet specification

### Multisorb Technologies Technical Data Sheet

**DATE:** 11/10/11

**PART NUMBER:** 02-01403CG02

**PRODUCT NAME:** FreshPax®, D-30cc, oxygen absorbing packet.

**DESCRIPTION:** FreshPax®, 30cc Type D oxygen absorbers are designed for modified atmosphere packaging of moist products with water activity less than .7, intended for storage and distribution at ambient or refrigerated temperatures down to 30 degrees F. When needed, the D-30cc FreshPax® will absorb its full capacity in 72-96 hours at ambient temperatures.

**PHYSICAL ATTRIBUTES:** 1.00 wide  $\pm$  0.03 inch X 1.75 long  $\pm$  0.07inch.  
(25.40 wide  $\pm$  0.76mm X 44.45 long  $\pm$  1.78mm)

**MATERIALS:** The face material is reverse printed, microperforated and bonded to an oil and grease resistant medium with a heat seal layer on the inside.

**PRINTING:** Red and blue print will include:  
DO NOT EAT appears on the artwork along with the EU  
do not eat symbol.

**PACKAGING:** The product will be as follows:

- 150 pieces/barrier pouch.
- 36 pouches per case.
- Total: 5400 pieces/case.
- Product label contains following:
  - Manufacturer's name
  - Description of product
  - Quantity per container
  - Manufacturer's part number
  - Manufacturer's control number

**PRODUCT STORAGE:** Cool Dry Location  
Best if used by date imprinted on label

All Statements, technical information and recommendations herein are based on tests we believe to be reliable, but the accuracy and completeness thereof is not guaranteed, and the following is made in lieu of all warranties expressed or implied, including the implied warranties of merchantability and fitness for purpose: Seller's and manufacturer's only obligation shall be to replace such quantity of the product proved to be defective. Before using, user shall determine the suitability of the product for its intended use, and user assumes all risk and liability whatsoever in connection therewith. Neither seller nor manufacturer shall be liable either in tort or in contract for any loss or damage, direct, or incidental, or consequential, arising out of the use of or the inability to use the product. No statement or recommendation not contained herein shall have any force or effect unless in an agreement signed by officers of seller and manufacturer. This information is the property of Multisorb Technologies and can only be revised or modified by Multisorb Technologies.

# FreshPax®

## Oxygen Absorbing Packets and Strips

### FreshPax Oxygen Absorbers vs. Ordinary Technologies

FreshPax packets are designed to be used in combination with your current packaging methods including gas flushing and vacuum packaging. Unlike gas flushing and vacuum packaging, which merely serve to dilute oxygen, FreshPax modifies package atmospheres by creating very low residual oxygen not normally achievable by these methods.

Packaging Method	Results	Benefit
FreshPax	Reduces and maintains Oxygen content in packaging to below 0.01%	Eliminates Aerobic Microbial Growth and Oxidative Chemical Reactions in Packaging
Vacuum and Back Flush	Achieves as little as 0.1% Residual Oxygen	Controls Microbial Growth Temporarily*
Gas Flushing	Approximately 0.5% to 5% Oxygen	Provides Some Control of Aerobic Microbial Growth*

\*Mold has been shown to grow in 20 days at 25°C at 0.2% residual oxygen (From "Techniques for the Preservation of Food by Employment of an Oxygen Absorber," Nakamura and Hoshino, 1963)

FreshPax Chemistry	Ideal Applications	Water Activity (estimated)	De-oxygenation Time Range*	Maximum Exposure to Air	Packet Sizes Available (cc of O <sub>2</sub> Absorbed)
Type B	Moist or semi-moist foods	>0.70	1-3 days	8 hours	(Packets or Strips) 10, 30, 50, 90, 100, 200, 300, 400, 500, 800, 750, 1000, 2000
Type D	Dehydrated or dry foods	<0.70	0.5 to 4 days	2 hours	(Packets or Strips) 25, 50, 100, 200, 300, 400, 500, 600, 750, 1000, 2000
Type R	Refrigerated or when rapid deoxygenation is required	All	8 hours to 2 days - call for specifics	1 hour	50, 100, 200, 300, 400, 500, 600, 750, 1000, 2000
Type M	Moist or semi-moist foods where a carbon dioxide flush has been used	>0.65	50% to 60% of capacity in 2-5 days. Full line capacity in 4-7 days.	1/2 day	20, 50, 100, 200, 400, 1000

**IMPORTANT NOTICE:** Manufacturer/Seller (collectively "Seller") disclaims any warranty for the product sample ("Sample") which Seller provides "AS IS" without warranty of any kind, either express or implied, including without limitation, the implied warranties of merchantability, fitness for a particular purpose or non-infringement. Seller does not guarantee the accuracy or completeness of any statements made regarding the Sample. User assumes all risk and liability in using the Sample. SELLER SHALL NOT BE LIABLE IN TORT OR CONTRACT FOR ANY LOSS OR DAMAGE, DIRECT, INCIDENTAL, OR CONSEQUENTIAL, ARISING OUT OF THE USE OF OR INABILITY TO USE THE SAMPLE. USER agrees to defend and indemnify Seller from all claims, liabilities, and costs (including attorneys' fees) related to User's use of the Sample. User is prohibited from re-selling Sample.

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### Package Requirements for successful use of FreshPax

FreshPax has been found to be effective when used with many kinds of packaging materials including EVOH and PVDC. Some important considerations to keep in mind regarding certain types of packaging include:

- Adequate barrier should be used – Plastic film must be checked for its oxygen permeability. A good target is to use a barrier material transmitting <15 cc of oxygen/M2/24 hours or less.
- Hermetic seals are essential – Non-hermetic seals reduce shelf life by using up oxygen absorber capacity prematurely.
- Package geometry – The package must be designed so as to allow free circulation of air around the product.

It is recommended that application tests with actual materials and equipment be conducted before using and specifying FreshPax. This includes determining the water activity of the product as nearly as possible, the packaging and distribution environment conditions, and specifications of the packaging film.



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# D.7 L\*a\*b\* raw data test 4

WEEK 1 June 18-22, 2012						
Date Collected	6/18/12 Day		3			
POS Building	Cooler A		Cooler B			
Sample Number	Control 7	Control 14	Test 7	Test 14		
Cooler Observations	Pink	Lighter pink	Discolored - brown/g	Pink		
R&D Lab	Control 7	Control 14	Test 7	Test 14		
MOCON - CO2 (%)	20.7	19.6	0.0	20.3		
MOCON - O2 (%)	0.080	0.149	21.700	0.020		
AVERAGE	Control	Test				
MOCON - CO2 (%)	20.15		10.15			
MOCON - O2 (%)	0.11		10.86			
L* (1)	56.70	60.76	60.23	61.05		
a* (1)	17.85	16.73	11.59	16.95		
b* (1)	8.27	9.09	8.78	8.33		
ΔL* (1)	0.46	4.52	3.99	4.81		
Δa* (1)	-0.34	-1.46	-6.60	-1.23		
Δb* (1)	0.16	0.97	0.66	0.21		
ΔE* (1)	0.59	4.84	7.74	4.97		
L* (2)	56.13	61.06	61.54	61.02		
a* (2)	18.38	16.38	10.56	17.00		
b* (2)	7.73	8.22	8.28	8.41		
ΔL* (2)	-0.11	4.81	5.30	4.78		
Δa* (2)	0.19	-1.80	-7.62	-1.18		
Δb* (2)	-0.39	0.10	0.16	0.29		
ΔE* (2)	0.45	5.14	9.29	4.93		
L* (3)	58.02	61.00	62.31	59.35		
a* (3)	17.72	16.16	11.01	18.02		
b* (3)	8.50	7.85	8.51	8.47		
ΔL* (3)	1.78	4.76	6.06	3.11		
Δa* (3)	-0.46	-2.02	-7.18	-0.17		
Δb* (3)	0.38	-0.27	0.39	0.35		
ΔE* (3)	1.88	5.18	9.41	3.14		
L* AVERAGE	56.95	60.94	61.36	60.47		
a* AVERAGE	17.98	16.42	11.05	17.32		
b* AVERAGE	8.17	8.39	8.52	8.40		
ΔL* AVERAGE	0.71	4.70	5.12	4.23		
Δa* AVERAGE	-0.20	-1.76	-7.13	-0.86		
Δb* AVERAGE	0.05	0.27	0.40	0.28		
ΔE* AVERAGE	0.97	5.05	8.81	4.35		
AVERAGE CONTROL	AVERAGE TEST					
L* AVERAGE	58.95		60.92			
a* AVERAGE	17.20		14.19			
b* AVERAGE	8.28		8.46			
ΔE* AVERAGE	3.01		6.58			
Observations						
Date Collected	6/20/12 Day		5			
POS Building	Cooler A		Cooler B			
Sample Number	Control 6	Control 13	Test 6	Test 13		
Cooler Observations						
R&D Lab	Control 6	Control 13	Test 6	Test 13		
MOCON - CO2 (%)	22.7	22.7	22.2	21.6		
MOCON - O2 (%)	0.094	0.048	0.000	0.000		
AVERAGE	Control	Test				
MOCON - CO2 (%)	22.70		21.90			
MOCON - O2 (%)	0.07		0.00			
L* (1)	58.32	61.51	59.99	57.05		
a* (1)	18.40	16.01	17.35	19.37		
b* (1)	9.29	8.06	8.42	8.07		
ΔL* (1)	-0.45	2.74	1.21	-1.73		
Δa* (1)	-0.02	-2.40	-1.06	0.96		
Δb* (1)	0.55	-0.69	-0.32	-0.68		
ΔE* (1)	0.71	3.70	1.64	2.09		
L* (2)	58.15	61.85	60.18	57.72		
a* (2)	18.49	15.96	17.73	19.05		
b* (2)	8.37	8.04	8.73	7.79		
ΔL* (2)	-0.63	3.07	1.40	-1.05		
Δa* (2)	0.07	-2.45	-0.69	0.64		
Δb* (2)	-0.37	-0.71	-0.02	-0.95		
ΔE* (2)	0.73	3.99	1.56	1.56		
L* (3)	58.15	60.97	59.85	57.97		
a* (3)	18.00	16.60	17.78	18.66		
b* (3)	8.55	8.54	8.16	7.68		
ΔL* (3)	-0.62	2.20	1.07	-0.80		
Δa* (3)	-0.41	-1.81	-0.63	0.25		
Δb* (3)	-0.19	-0.20	-0.58	-1.06		
ΔE* (3)	0.77	2.86	1.37	1.35		
L* AVERAGE	58.21	61.44	60.01	57.58		
a* AVERAGE	18.30	16.19	17.62	19.03		
b* AVERAGE	8.74	8.21	8.44	7.85		
ΔL* AVERAGE	-0.57	2.67	1.23	-1.19		
Δa* AVERAGE	-0.12	-2.22	-0.79	0.62		
Δb* AVERAGE	0.00	-0.53	-0.31	-0.90		
ΔE* AVERAGE	0.74	3.52	1.52	1.67		
AVERAGE CONTROL	AVERAGE TEST					
L* AVERAGE	59.83		58.79			
a* AVERAGE	17.24		18.32			
b* AVERAGE	8.48		8.14			
ΔE* AVERAGE	2.13		1.60			
Observations						
Date frozen control was removed from freezer	6/19/2012					
Date Collected	6/22/12 Day		7			
POS Building	Cooler A		Cooler B			
Sample Number	Control 5	Control 12	Test 5	Test 12		
Cooler Observations	Food in seal					
R&D Lab	Control 5	Control 12	Test 5	Test 12		
MOCON - CO2 (%)	23.1	4.1	22.4	22.0		
MOCON - O2 (%)	0.084	19.700	0.000	0.000		
AVERAGE	Control	Test				
MOCON - CO2 (%)	13.60		22.20			
MOCON - O2 (%)	9.87		0.00			
L* (1)	58.07	60.99	59.84	58.88		
a* (1)	18.09	11.27	17.81	18.16		
b* (1)	8.68	11.05	8.84	7.96		
ΔL* (1)	-0.19	2.72	1.58	0.61		
Δa* (1)	0.10	-7.32	-0.78	-0.43		
Δb* (1)	-0.60	1.77	-0.44	-1.32		
ΔE* (1)	0.64	8.00	1.82	1.52		
L* (2)	57.54	61.14	60.05	58.49		
a* (2)	18.85	12.08	17.94	18.75		
b* (2)	9.01	10.92	9.20	8.21		
ΔL* (2)	-0.72	2.88	1.78	0.23		
Δa* (2)	0.27	-6.51	-0.64	0.16		
Δb* (2)	-0.27	1.64	-0.09	-1.08		
ΔE* (2)	0.81	7.30	1.90	1.11		
L* (3)	58.11	60.55	59.79	58.54		
a* (3)	18.71	13.18	18.24	19.29		
b* (3)	8.97	10.23	9.60	8.87		
ΔL* (3)	-0.16	2.29	1.53	-0.63		
Δa* (3)	0.13	-5.40	-0.35	0.71		
Δb* (3)	-0.31	0.94	0.32	-0.42		
ΔE* (3)	0.37	5.94	1.60	1.01		
L* AVERAGE	57.91	60.89	59.89	58.33		
a* AVERAGE	18.75	12.18	18.00	18.73		
b* AVERAGE	8.89	10.73	9.21	8.35		
ΔL* AVERAGE	-0.36	2.63	1.63	0.07		
Δa* AVERAGE	0.17	-6.41	-0.59	0.35		
Δb* AVERAGE	-0.39	1.45	-0.07	-0.94		
ΔE* AVERAGE	0.61	7.08	1.77	1.22		
AVERAGE CONTROL	AVERAGE TEST					
L* AVERAGE	59.40		59.11			
a* AVERAGE	15.46		18.37			
b* AVERAGE	9.81		8.76			
ΔE* AVERAGE	3.84		1.50			
Observations						
Date frozen control was removed from freezer	6/21/2012					

WEEK 2 June 25-29, 2012				
Date Collected	6/25/12 Day		10 Measured on	
R&D Lab	Control 4	Control 11	Test 4	Test 11
MOCON - CO2 (%)	23.1	22.2	22.1	21.0
MOCON - O2 (%)	0.103	0.131	0.000	0.000
AVERAGE	Control	Test		
MOCON - CO2 (%)	22.65		21.55	
MOCON - O2 (%)	0.12		0.00	
	Control 4	Control 11	Test 4	Test 11
L* (1)	60.82	56.99	59.24	58.24
a* (1)	17.51	20.74	17.94	19.35
b* (1)	9.13	9.03	8.97	8.76
ΔL* (1)	0.60	-3.23	-0.99	-1.99
Δa* (1)	0.04	3.27	0.47	1.88
Δb* (1)	-0.33	-0.42	-0.48	-0.69
ΔE* (1)	0.69	4.61	1.20	2.82
L* (2)	60.29	59.23	60.04	59.23
a* (2)	17.33	18.56	17.86	18.92
b* (2)	10.00	7.87	8.13	7.95
ΔL* (2)	0.06	-1.00	-0.19	-1.00
Δa* (2)	-0.14	1.09	0.39	1.45
Δb* (2)	0.54	-1.59	-1.32	-1.50
ΔE* (2)	0.56	2.17	1.39	2.31
L* (3)	59.35	61.02	60.48	58.96
a* (3)	17.54	17.25	18.29	19.04
b* (3)	9.83	8.15	7.79	8.14
ΔL* (3)	-0.88	0.79	0.25	-1.27
Δa* (3)	-0.06	-0.22	0.82	1.57
Δb* (3)	0.37	-1.31	-1.66	-1.32
ΔE* (3)	0.96	1.54	1.87	2.41
L* AVERAGE	60.15	59.08	59.92	58.81
a* AVERAGE	17.46	18.85	18.03	19.10
b* AVERAGE	9.65	8.35	8.30	8.28
ΔL* AVERAGE	-0.07	-1.15	-0.31	-1.42
Δa* AVERAGE	-0.05	1.38	0.56	1.63
Δb* AVERAGE	0.19	-1.11	-1.15	-1.17
ΔE* AVERAGE	0.74	2.77	1.49	2.51
	AVERAGE CONTROL	AVERAGE TEST		
L* AVERAGE	59.62	59.37		
a* AVERAGE	18.16	18.57		
b* AVERAGE	9.00	8.29		
ΔE* AVERAGE	1.76	2.00		
Observations				
Date Collected	6/27/12 Day		12 Measured on	
POS Building	Cooler A		Cooler B	
Sample Number	Control 3	Control 10	Test 3	Test 10
Cooler Observations				
R&D Lab	Control 19	Control 39	Test 19	Test 39
MOCON - CO2 (%)	22.9	22.5	21.5	22.2
MOCON - O2 (%)	0.122	0.072	0.000	0.064
AVERAGE	Control	Test		
MOCON - CO2 (%)	22.70		21.85	
MOCON - O2 (%)	0.10		0.03	
	Control 19	Control 39	Test 19	Test 39
L* (1)	58.94	57.11	54.95	61.35
a* (1)	18.04	18.04	20.32	17.32
b* (1)	9.52	8.35	10.06	9.77
ΔL* (1)	-0.22	-2.05	-4.20	2.19
Δa* (1)	0.19	0.20	2.47	-0.52
Δb* (1)	-0.02	-1.19	0.51	0.23
ΔE* (1)	0.29	2.38	4.90	2.26
L* (2)	58.88	56.51	55.03	61.18
a* (2)	17.84	18.04	20.00	17.43
b* (2)	9.31	9.01	9.65	9.62
ΔL* (2)	-0.27	-2.64	-4.13	2.03
Δa* (2)	0.00	0.19	2.16	-0.41
Δb* (2)	-0.23	-0.54	0.10	0.07
ΔE* (2)	0.36	2.70	4.66	2.07
L* (3)	58.72	56.15	56.76	60.40
a* (3)	17.79	18.61	18.97	18.23
b* (3)	8.68	8.73	8.52	9.22
ΔL* (3)	-0.44	-3.01	-2.39	1.24
Δa* (3)	-0.05	0.76	1.13	0.39
Δb* (3)	-0.86	-0.81	-1.03	-0.33
ΔE* (3)	0.97	3.21	2.84	1.34
L* AVERAGE	58.85	56.59	55.58	60.98
a* AVERAGE	17.89	18.23	19.76	17.66
b* AVERAGE	9.17	8.70	9.41	9.54
ΔL* AVERAGE	-0.31	-2.57	-3.57	1.82
Δa* AVERAGE	0.05	0.38	1.92	-0.18
Δb* AVERAGE	-0.37	-0.85	-0.14	-0.01
ΔE* AVERAGE	0.54	2.76	4.13	1.89
	AVERAGE CONTROL	AVERAGE TEST		
L* AVERAGE	57.72	58.28		
a* AVERAGE	18.06	18.71		
b* AVERAGE	8.93	9.47		
ΔE* AVERAGE	1.65	3.01		
Observations				
Observations	Date frozen control was removed from freezer			
	6/28/2012			

WEEK 3 July 2-6, 2012				
Date Collected	7/2/12	Day	17	Measured on
<i>R&amp;D Lab</i>	Control 18	Control 38	Test 18	Test 38
MOCON - CO2 (%)	23.9	22.8	22.2	21.7
MOCON - O2 (%)	0.076	0.063	0.000	0.000
<b>AVERAGE</b>	<b>Control</b>		<b>Test</b>	
MOCON - CO2 (%)	23.35		21.95	
MOCON - O2 (%)	0.07		0.00	
	Control 18	Control 38	Test 18	Test 38
L* (1)	58.13	63.73	57.53	57.10
a* (1)	18.75	15.43	19.35	19.43
b* (1)	9.69	9.48	8.90	8.34
ΔL* (1)	-0.26	5.33	-0.87	-1.30
Δa* (1)	0.29	-3.03	0.89	0.97
Δb* (1)	1.43	1.22	0.64	0.08
ΔE* (1)	1.49	6.25	1.40	1.63
L* (2)	57.84	63.14	58.66	56.61
a* (2)	18.51	16.47	19.20	19.20
b* (2)	8.81	9.52	7.91	8.95
ΔL* (2)	-0.56	4.74	0.26	-1.79
Δa* (2)	0.05	-1.99	0.74	0.74
Δb* (2)	0.55	1.26	-0.35	0.69
ΔE* (2)	0.79	5.29	0.86	2.06
L* (3)	58.14	60.51	58.46	56.88
a* (3)	18.19	18.17	19.04	19.44
b* (3)	8.28	9.51	8.11	8.52
ΔL* (3)	-0.26	2.11	0.06	-1.52
Δa* (3)	-0.27	-0.29	0.53	0.98
Δb* (3)	0.02	1.25	-0.15	0.26
ΔE* (3)	0.37	2.47	0.60	1.83
<b>L* AVERAGE</b>	58.04	62.46	58.22	56.86
<b>a* AVERAGE</b>	18.48	16.69	19.20	19.36
<b>b* AVERAGE</b>	8.93	9.50	8.31	8.60
<b>ΔL* AVERAGE</b>	-0.36	4.06	-0.18	-1.54
<b>Δa* AVERAGE</b>	0.02	-1.77	0.72	0.90
<b>Δb* AVERAGE</b>	0.67	1.24	0.05	0.34
<b>ΔE* AVERAGE</b>	0.88	4.67	0.95	1.84
		<b>AVERAGE CONTROL</b>		<b>AVERAGE TEST</b>
<b>L* AVERAGE</b>		60.25		57.54
<b>a* AVERAGE</b>		17.59		19.28
<b>b* AVERAGE</b>		9.22		8.46
<b>ΔE* AVERAGE</b>		2.78		1.40
Observations				

WEEK 4 July 9-16, 2012					Date Collected	7/13/12	Day	28	Measured on
Date Collected	7/9/12	Day	24	Measured on	R&D Lab	Control 24	Control 31	Test 24	Test 31
R&D Lab	Control 26	Control 33	Test 26	Test 33	MOCON - CO2 (%)	20.5	21.4	21.0	19.9
Observations	Visible gray line on cut edges.			Sachet was present	MOCON - O2 (%)	0.083	0.025	0.000	0.000
MOCON - CO2 (%)	22.8	23.3	21.0	19.2	AVERAGE	Control		Test	
MOCON - O2 (%)	0.071	0.044	0.000	15.300	MOCON - CO2 (%)	20.95		20.45	
AVERAGE	Control		Test		MOCON - O2 (%)	0.05		0.00	
MOCON - CO2 (%)	23.05		20.10		Control 24	Control 31	Test 24	Test 31	
MOCON - O2 (%)	0.06		7.65		L* (1)	57.05	59.17	59.42	60.78
L* (1)	56.81	61.59	60.65	60.52	a* (1)	18.62	19.81	17.90	16.83
a* (1)	18.71	16.97	17.49	17.80	b* (1)	9.40	10.91	9.27	8.93
b* (1)	8.40	8.68	10.09	9.91	ΔL* (1)	0.28	2.40	2.65	4.00
ΔL* (1)	-0.89	3.88	2.94	2.82	Δa* (1)	-0.29	0.90	-1.01	-2.09
Δa* (1)	0.83	-0.91	-0.40	-0.60	Δb* (1)	-0.17	1.33	-0.31	-0.64
Δb* (1)	-0.91	-0.63	0.77	0.59	ΔE* (1)	0.44	2.89	2.85	4.56
ΔE* (1)	1.52	4.04	3.07	2.94	L* (2)	56.50	60.79	58.70	60.85
L* (2)	57.95	62.06	60.64	60.66	a* (2)	19.20	19.19	18.36	16.87
a* (2)	17.88	16.66	17.77	17.61	b* (2)	9.20	9.74	9.55	8.97
b* (2)	8.74	9.84	9.21	9.56	ΔL* (2)	-0.28	4.02	1.93	4.07
ΔL* (2)	0.25	4.35	2.94	2.95	Δa* (2)	0.29	0.28	-0.56	-2.05
Δa* (2)	0.00	-1.22	-0.11	-0.27	Δb* (2)	-0.39	0.17	-0.02	-0.61
Δb* (2)	-0.58	0.53	-0.11	0.25	ΔE* (2)	0.55	4.03	2.01	4.60
ΔE* (2)	0.63	4.55	2.94	2.97	L* (3)	56.27	60.15	58.74	60.50
L* (3)	58.07	59.81	59.44	60.51	a* (3)	19.37	19.58	18.46	16.97
a* (3)	17.29	18.37	18.18	17.99	b* (3)	9.26	11.24	9.07	9.31
b* (3)	8.91	9.93	8.90	8.80	ΔL* (3)	-0.51	3.38	1.96	3.73
ΔL* (3)	0.37	2.10	1.73	2.81	Δa* (3)	0.45	0.66	-0.46	-1.95
Δa* (3)	-0.60	0.49	0.29	0.11	Δb* (3)	-0.31	1.67	-0.50	-0.26
Δb* (3)	-0.40	0.62	-0.41	-0.51	ΔE* (3)	0.75	3.82	2.08	4.21
ΔE* (3)	0.81	2.24	1.80	2.86	L* AVERAGE	56.61	60.04	58.95	60.71
L* AVERAGE	57.61	61.15	60.24	60.56	a* AVERAGE	19.06	19.53	18.24	16.89
a* AVERAGE	17.96	17.33	17.81	17.80	b* AVERAGE	9.29	10.63	9.30	9.07
b* AVERAGE	8.68	9.48	9.40	9.42	ΔL* AVERAGE	-0.17	3.27	2.18	3.93
ΔL* AVERAGE	-0.09	3.44	2.54	2.86	Δa* AVERAGE	0.15	0.61	-0.68	-2.03
Δa* AVERAGE	0.08	-0.55	-0.07	-0.25	Δb* AVERAGE	-0.29	1.06	-0.28	-0.50
Δb* AVERAGE	-0.63	0.17	0.08	0.11	ΔE* AVERAGE	0.58	3.58	2.31	4.46
ΔE* AVERAGE	0.99	3.61	2.60	2.92		AVERAGE CONTROL		AVERAGE TEST	
		AVERAGE CONTROL	AVERAGE TEST		L* AVERAGE	58.32		59.83	
L* AVERAGE		59.38	60.40		a* AVERAGE	19.30		17.57	
a* AVERAGE		17.65	17.81		b* AVERAGE	9.96		9.18	
b* AVERAGE		9.08	9.41		ΔE* AVERAGE	2.08		3.39	
ΔE* AVERAGE		2.30	2.76		Observations				
Observations									
Date Collected	7/16/12	Day	31	Measured on	R&D Lab	Control 23	Control 30	Test 23	Test 30
Date Collected	7/11/12	Day	26	Measured on	MOCON - CO2 (%)	20.4	20.6	20.6	20.6
R&D Lab	Control 25	Control 32	Test 25	Test 32	MOCON - O2 (%)	0.046	0.067	0.000	0.000
MOCON - CO2 (%)	22.7	22.9	22.7	21.8	AVERAGE	Control		Test	
MOCON - O2 (%)	0.059	0.062	0.000	0.000	MOCON - CO2 (%)	20.50		20.60	
AVERAGE	Control		Test		MOCON - O2 (%)	0.06		0.00	
MOCON - CO2 (%)	22.80		22.25		Control 23	Control 30	Test 23	Test 30	
MOCON - O2 (%)	0.06		0.00		L* (1)	59.30	58.56	57.53	55.91
L* (1)	59.31	58.16	59.46	59.78	a* (1)	18.02	18.36	18.53	19.42
a* (1)	17.63	18.00	18.70	17.53	b* (1)	9.11	9.53	9.07	8.68
b* (1)	10.38	9.52	9.19	7.84	ΔL* (1)	-0.03	-0.77	-1.80	-3.43
ΔL* (1)	0.49	0.67	0.63	0.96	Δa* (1)	0.06	0.40	0.56	1.45
Δa* (1)	-0.63	-0.25	0.45	-0.73	Δb* (1)	-0.38	0.04	-0.42	-0.81
Δb* (1)	1.42	0.56	0.23	-1.12	ΔE* (1)	0.39	0.87	1.94	3.81
ΔE* (1)	1.63	0.91	0.81	1.64	L* (2)	59.23	59.24	57.63	55.15
L* (2)	58.42	57.62	59.93	60.77	a* (2)	18.15	18.07	18.42	19.81
a* (2)	18.32	18.29	18.59	16.78	b* (2)	9.25	9.17	8.75	8.33
b* (2)	9.81	9.00	9.01	7.35	ΔL* (2)	-0.10	-0.10	-1.70	-4.19
ΔL* (2)	-0.41	-1.20	1.11	1.45	Δa* (2)	0.18	0.10	0.46	1.84
Δa* (2)	0.06	0.03	0.33	-1.47	Δb* (2)	-0.24	-0.32	-0.74	-1.16
Δb* (2)	0.85	0.04	0.05	-1.61	ΔE* (2)	0.32	0.35	1.91	4.72
ΔE* (2)	0.95	1.20	1.16	2.62	L* (3)	59.10	58.48	57.54	55.38
L* (3)	58.30	57.83	59.96	60.24	a* (3)	17.99	18.24	18.32	19.96
a* (3)	18.66	18.58	18.26	17.09	b* (3)	9.24	8.94	9.00	8.15
b* (3)	9.60	8.65	9.46	7.52	ΔL* (3)	-0.23	-0.86	-1.80	-3.96
ΔL* (3)	-0.53	-0.99	1.14	1.42	Δa* (3)	0.02	0.27	0.35	2.00
Δa* (3)	0.40	0.32	0.00	-1.17	Δb* (3)	-0.25	-0.55	-0.49	-1.34
Δb* (3)	0.64	-0.31	0.50	-1.44	ΔE* (3)	0.34	1.06	1.90	4.63
ΔE* (3)	0.92	1.09	1.25	2.34	L* AVERAGE	59.21	58.76	57.57	55.48
L* AVERAGE	58.68	57.87	59.78	60.10	a* AVERAGE	18.05	18.22	18.42	19.73
a* AVERAGE	18.20	18.29	18.52	17.13	b* AVERAGE	9.20	9.21	8.94	8.39
b* AVERAGE	9.93	9.06	9.22	7.57	ΔL* AVERAGE	-0.12	-0.58	-1.77	-3.86
ΔL* AVERAGE	-0.15	-0.51	0.96	1.28	Δa* AVERAGE	0.09	0.26	0.46	1.76
Δa* AVERAGE	-0.06	0.03	0.26	-1.12	Δb* AVERAGE	-0.29	-0.28	-0.55	-1.10
Δb* AVERAGE	0.97	0.10	0.26	-1.39	ΔE* AVERAGE	0.35	0.76	1.92	4.39
ΔE* AVERAGE	1.17	1.07	1.07	2.20		AVERAGE CONTROL		AVERAGE TEST	
		AVERAGE CONTROL	AVERAGE TEST		L* AVERAGE	58.27		56.52	
L* AVERAGE		58.27	59.94		a* AVERAGE	18.25		19.08	
a* AVERAGE		18.25	17.83		b* AVERAGE	9.49		8.66	
b* AVERAGE		9.49	8.40		ΔE* AVERAGE	1.12		3.15	
ΔE* AVERAGE		1.12	1.64		Observations				
Observations					Observations				

Date Collected	7/16/12	Day	PHOTO SAMPLE	Measured on
Observations	Faded		Also faded but more pink than	
R&D Lab	Control 20	Control 41	Test 20	Test 41
MOCON - CO2 (%)	21.4	21.4	20.3	20.6
MOCON - O2 (%)	0.034	0.012	0.000	0.000
AVERAGE	Control		Test	
MOCON - CO2 (%)	21.40		20.45	
MOCON - O2 (%)	0.02		0.00	
	Control 20	Control 41	Test 20	Test 41
L* (1)	60.37	62.48	57.00	58.01
a* (1)	16.75	15.26	18.64	18.96
b* (1)	9.28	8.70	8.63	9.11
ΔL* (1)	-0.89	1.22	-4.25	-3.24
Δa* (1)	0.49	-1.00	2.38	2.70
Δb* (1)	-0.21	-0.79	-0.86	-0.38
ΔE* (1)	1.04	1.76	4.95	4.24
L* (2)	61.10	61.62	56.97	57.60
a* (2)	16.36	15.61	18.53	19.19
b* (2)	9.49	8.30	8.77	9.26
ΔL* (2)	-0.15	0.37	-4.28	-3.66
Δa* (2)	0.09	-0.65	2.27	2.93
Δb* (2)	0.00	-1.19	-0.72	-0.23
ΔE* (2)	0.18	1.41	4.90	4.69
L* (3)	60.84	59.87	57.34	57.20
a* (3)	16.65	16.71	18.66	19.48
b* (3)	9.15	8.33	8.95	9.23
ΔL* (3)	-0.42	-1.39	-3.91	-4.06
Δa* (3)	0.38	0.44	2.39	3.21
Δb* (3)	-0.34	-1.16	-0.54	-0.26
ΔE* (3)	0.66	1.86	4.62	5.18
L* AVERAGE	60.77	61.32	57.10	57.60
a* AVERAGE	16.59	15.86	18.61	19.21
b* AVERAGE	9.31	8.44	8.78	9.20
ΔL* AVERAGE	-0.49	0.07	-4.15	-3.65
Δa* AVERAGE	0.32	-0.40	2.35	2.95
Δb* AVERAGE	-0.18	-1.05	-0.71	-0.29



## Appendix E – Test 5 –Use of ultraviolet (UV) films to control photooxidation, oxygen scavenger revisited

**E.1** Visual appearance of the control samples compared to the UV films and scavenger treatments at day 4. Discoloration is only evident on the scavenger sample (S6) that had high oxygen, with some fading of control cooler A sample (C6) which is made more evident by the brighter pink color of the ham that was covered by the label. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



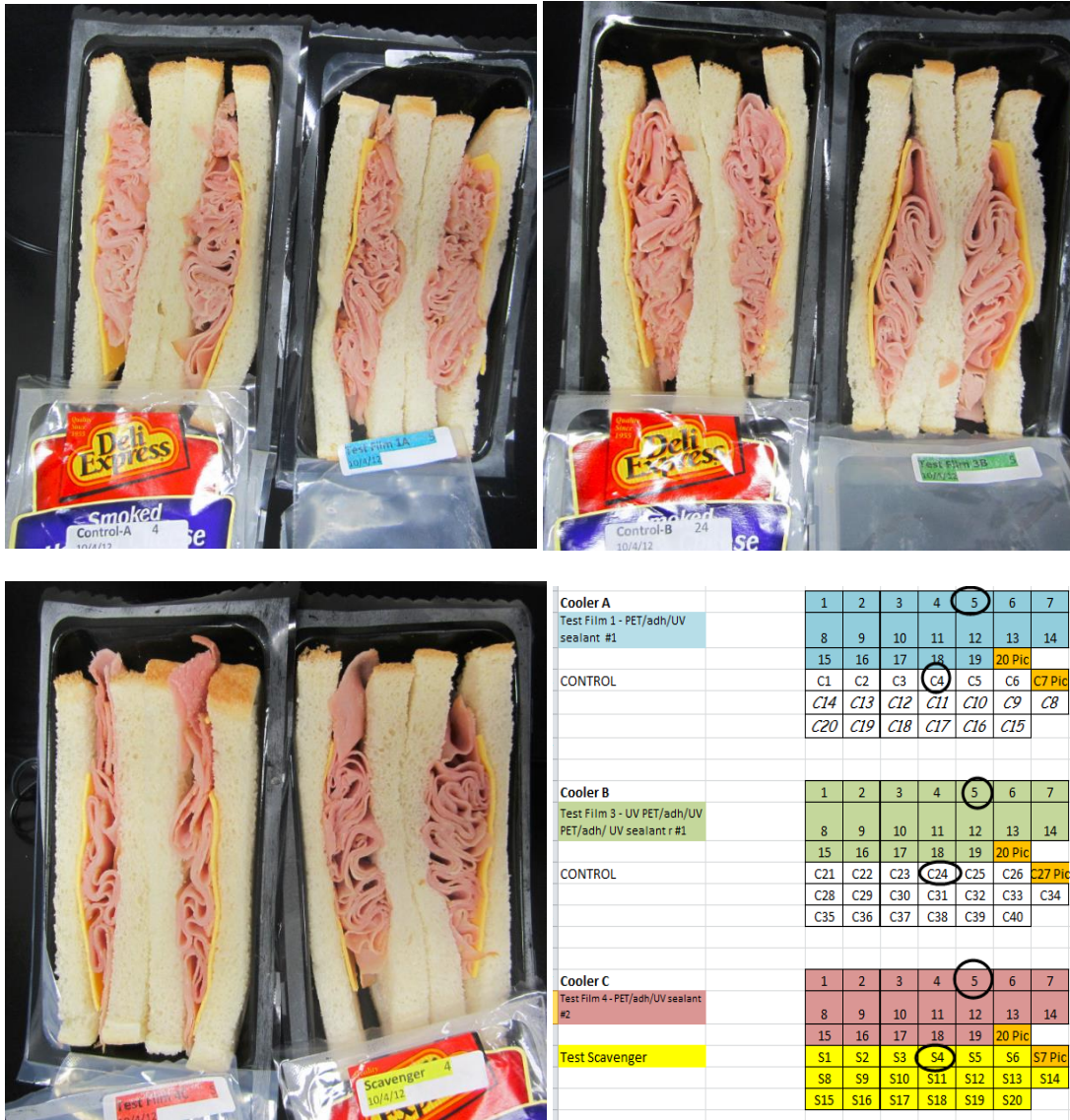
Cooler A Test Film 1 - PET/adh/UV sealant #1	1	2	3	4	5	6	7
	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
	C1	C2	C3	C4	C5	C6	C7 Pic
	C14	C13	C12	C11	C10	C9	C8
Cooler B Test Film 3 - UV PET/adh/UV PET/adh/ UV sealant r #1	1	2	3	4	5	6	7
	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
	C21	C22	C23	C24	C25	C26	C27 Pic
	C28	C29	C30	C31	C32	C33	C34
Cooler C Test Film 4 - PET/adh/UV sealant #2	1	2	3	4	5	6	7
	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
	S1	S2	S3	S4	S5	S6	S7 Pic
	S8	S9	S10	S11	S12	S13	S14
Test Scavenger	S15	S16	S17	S18	S19	S20	

E.2 Visual appearance of the control samples compared to the UV film and scavenger treatments at day 6. Fading is detected on all samples except UV test film 3 and the scavenger sample (which was missing the sachet, so in effect was a control package). Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.

	1	2	3	4	5	6	7
<b>Cooler A</b>							
Test Film 1 - PET/adh/UV sealant #1	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
CONTROL	C1	C2	C3	C4	C5	C6	C7 Pic
	C14	C13	C12	C11	C10	C9	C8
	C20	C19	C18	C17	C16	C15	
<b>Cooler B</b>							
Test Film 3 - UV PET/adh/UV PET/adh/ UV sealant r #1	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
CONTROL	C21	C22	C23	C24	C25	C26	C27 Pic
	C28	C29	C30	C31	C32	C33	C34
	C35	C36	C37	C38	C39	C40	
<b>Cooler C</b>							
Test Film 4 - PET/adh/UV sealant #2	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
Test Scavenger	S1	S2	S3	S4	S5	S6	S7 Pic
	S8	S9	S10	S11	S12	S13	S14
	S15	S16	S17	S18	S19	S20	



E.3 Visual appearance of the control samples compared to the scavenger test samples at day 8. Discoloration is not evident in any treatment. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



E.4 Visual appearance of the control samples compared to the scavenger test samples at day 11. Some fading is detected in the control cooler A sample (C3). Other treatments do not appear to have discoloration or fading. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.

<b>Cooler A</b>		1	2	3	4	5	6	7
Test Film 1 - PET/adh/UV sealant #1		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
CONTROL		C1	C2	C3	C4	C5	C6	C7 Pic
		C14	C13	C12	C11	C10	C9	C8
		C20	C19	C18	C17	C16	C15	
<b>Cooler B</b>		1	2	3	4	5	6	7
Test Film 3 - UV PET/adh/UV PET/adh/ UV sealant r #1		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
CONTROL		C21	C22	C23	C24	C25	C26	C27 Pic
		C28	C29	C30	C31	C32	C33	C34
		C35	C36	C37	C38	C39	C40	
<b>Cooler C</b>		1	2	3	4	5	6	7
Test Film 4 - PET/adh/UV sealant #2		8	9	10	11	12	13	14
		15	16	17	18	19	20 Pic	
Test Scavenger		S1	S2	S3	S4	S5	S6	S7 Pic
		S8	S9	S10	S11	S12	S13	S14
		S15	S16	S17	S18	S19	S20	



E.5 Visual appearance of the control samples compared to the scavenger test samples at day 13. Discoloration is evident in all treatments with the exceptions of UV test film 1 (#3) and UV test film 4 (#3). However, these sandwiches were further from the light source in comparison. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



E.6 Visual appearance of the control samples compared to the scavenger test samples at day 15. Discoloration is evident in all treatments. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Cooler A	1	2	3	4	5	6	7
	Test Film 1 - PET/adh/UV sealant #1						14
	8	9	10	11	12	13	
	15	16	17	18	19	20 Pic	
	C1	C2	C3	C4	C5	C6	C7 Pic
CONTROL	C14	C13	C12	C11	C10	C9	C8
	C20	C19	C18	C17	C16	C15	
Cooler B	1	2	3	4	5	6	7
	Test Film 3 - UV PET/adh/UV PET/adh/ UV sealant r #1						14
	8	9	10	11	12	13	
	15	16	17	18	19	20 Pic	
	C21	C22	C23	C24	C25	C26	C27 Pic
CONTROL	C28	C29	C30	C31	C32	C33	C34
	C35	C36	C37	C38	C39	C40	
Cooler C	1	2	3	4	5	6	7
	Test Film 4 - PET/adh/UV sealant #2						14
	8	9	10	11	12	13	
	15	16	17	18	19	20 Pic	
	S1	S2	S3	S4	S5	S6	S7 Pic
Test Scavenger	S8	S9	S10	S11	S12	S13	S14
	S15	S16	S17	S18	S19	S20	



E.7 Visual appearance of the control samples compared to the scavenger test samples at day 32. Discoloration is evident in all treatments. Cooler location of the sample number is circled in the cooler layout below. The light source is on the right hand side of the cooler.



Date	10/24/2012						
Cooler A	1	2	3	4	5	6	7
Test Film 1 - PET/adh/UV sealant #1	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
CONTROL	C1	C2	C3	C4	C5	C6	C7 Pic
	C14	C13	C12	C11	C10	C9	C8
	C20	C19	C18	C17	C16	C15	
Cooler B	1	2	3	4	5	6	7
Test Film 3 - UV PET/adh/UV PET/adh/ UV sealant r #1	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
CONTROL	C21	C22	C23	C24	C25	C26	C27 Pic
	C28	C29	C30	C31	C32	C33	C34
	C35	C36	C37	C38	C39	C40	
Cooler C	1	2	3	4	5	6	7
Test Film 4 - PET/adh/UV sealant #2	8	9	10	11	12	13	14
	15	16	17	18	19	20 Pic	
Test Scavenger	S1	S2	S3	S4	S5	S6	S7 Pic
	S8	S9	S10	S11	S12	S13	S14
	S15	S16	S17	S18	S19	S20	

E.8  $L^*a^*b^*$  raw data (3 measurements are combine to be the representative  $L^*a^*b^*$  score for the sample).

<b>WEEK 1</b>						
Date Collected	10/15/12	Day	4			
<i>R&amp;D Lab</i>	Control A6	Test F1#7	Control B26	Test F3#7	Test F4#7	Scavenger #6
MOCON - CO2 (%)	16.8	19.4	16.8	18.9	17.3	12.1
MOCON - O2 (%)	0.435	0.389	0.354	0.427	0.335	7.890
	Control A6	Test F1#7	Control B26	Test F3#7	Test F4#7	Scavenger #6
L* (1)	57.70	59.50	62.39	60.41	62.44	
a* (1)	17.97	16.70	17.01	17.91	16.24	
b* (1)	8.70	8.57	8.20	8.90	9.07	
L* (2)	57.02	59.33	60.46	62.74	65.10	
a* (2)	18.42	16.71	18.01	16.85	14.37	
b* (2)	7.76	8.37	7.45	8.73	8.68	
L* (3)	57.77	58.68	60.86	63.67	65.56	
a* (3)	18.63	17.53	16.89	16.25	13.68	
b* (3)	8.40	8.57	8.15	8.51	8.08	
L* AVERAGE	57.50	59.17	61.24	62.27	64.37	
a* AVERAGE	18.34	16.98	17.30	17.00	14.76	
b* AVERAGE	8.29	8.50	7.93	8.71	8.61	
Date Collected	10/17/12	Day	6			
<i>R&amp;D Lab</i>	Control A5	Test F1#6	Control B25	Test F3#6	Test F4#6	Scavenger #5
MOCON - CO2 (%)	16.0	16.2	16.8	16.9	16.9	15.9
MOCON - O2 (%)	0.419	0.372	0.344	0.322	0.308	0.350
	Control A5	Test F1#6	Control B25	Test F3#6	Test F4#6	Scavenger #5
L* (1)	60.57	60.47	64.39	60.30	67.05	
a* (1)	18.29	16.66	16.12	17.11	14.21	
b* (1)	8.80	8.77	8.50	8.05	8.99	
L* (2)	61.40	60.17	64.48	60.96	68.04	
a* (2)	18.18	17.28	16.39	17.05	13.54	
b* (2)	8.57	8.34	8.45	7.97	8.65	
L* (3)	61.23	59.94	64.15	60.72	65.01	
a* (3)	17.81	17.33	16.52	17.25	19.94	
b* (3)	9.00	8.80	8.27	8.15	9.35	
L* AVERAGE	61.07	60.19	64.34	60.66	66.70	
a* AVERAGE	18.09	17.09	16.34	17.14	15.90	
b* AVERAGE	8.79	8.64	8.41	8.06	9.00	
Date Collected	10/19/12	Day	8			
<i>R&amp;D Lab</i>	Control A4	Test F1#5	Control B4	Test F3#5	Test F4#5	Scavenger 4
MOCON - CO2 (%)	17.6	17.3	16.2	16.6	16.4	17.8
MOCON - O2 (%)	0.399	0.314	0.336	0.359	0.396	0.347
	Control A4	Test F1#5	Control B4	Test F3#5	Test F4#5	Scavenger 4
L* (1)	62.78	59.22	59.74	63.35	58.24	
a* (1)	16.40	17.84	18.38	16.65	18.03	
b* (1)	8.07	8.57	8.18	8.65	6.46	
L* (2)	64.71	60.44	60.89	64.18	60.22	
a* (2)	15.41	17.61	17.62	15.78	17.11	
b* (2)	8.66	8.62	8.39	8.19	7.23	
L* (3)	64.04	60.22	60.41	64.08	61.74	
a* (3)	15.67	17.89	17.97	15.96	16.38	
b* (3)	8.06	8.68	8.37	8.16	6.82	
L* AVERAGE	63.84	59.96	60.35	63.87	60.07	
a* AVERAGE	15.83	17.78	17.99	16.13	17.17	
b* AVERAGE	8.26	8.62	8.31	8.33	6.84	



<b>WEEK 2</b>						
<b>Date Collected</b>	10/22/12 Day		11			
<i>R&amp;D Lab</i>	<b>Control A3</b>	<b>Test F1#4</b>	<b>Control B23</b>	<b>Test F3#4</b>	<b>Test F4#4</b>	<b>Scavenger #3</b>
MOCON - CO2 (%)	16.9	16.1	17.9	18.6	17.2	16.0
MOCON - O2 (%)	0.383	0.327	0.286	0.344	0.341	0.203
	<b>Control A3</b>	<b>Test F1#4</b>	<b>Control B23</b>	<b>Test F3#4</b>	<b>Test F4#4</b>	<b>Scavenger #3</b>
L* (1)	61.53	60.26	59.79	60.79	62.14	59.25
a* (1)	17.11	19.47	18.18	17.49	17.56	18.12
b* (1)	7.57	8.69	8.69	7.25	8.23	7.55
L* (2)	61.93	60.10	60.37	60.47	61.93	58.53
a* (2)	16.96	19.27	17.71	17.58	17.62	18.46
b* (2)	7.71	8.70	8.45	7.09	8.47	7.14
L* (3)	61.38	60.28	60.26	60.61	61.52	58.39
a* (3)	17.27	19.09	18.15	17.19	17.84	18.62
b* (3)	7.70	8.40	7.87	7.50	8.55	7.33
<b>L* AVERAGE</b>	61.61	60.21	60.14	60.62	61.86	58.72
<b>a* AVERAGE</b>	17.11	19.28	18.01	17.42	17.67	18.40
<b>b* AVERAGE</b>	7.66	8.60	8.34	7.28	8.42	7.34
<b>Date Collected</b>	10/24/12 Day		13			
<i>R&amp;D Lab</i>	<b>Control A8</b>	<b>Test F1#3</b>	<b>Control B34</b>	<b>Test F3#3</b>	<b>Test F4#3</b>	<b>Scavenger #14</b>
MOCON - CO2 (%)	16.9	16.8	17.0	17.2	16.5	17.4
MOCON - O2 (%)	0.263	0.346	0.285	0.340	0.332	0.285
	<b>Control A8</b>	<b>Test F1#3</b>	<b>Control B34</b>	<b>Test F3#3</b>	<b>Test F4#3</b>	<b>Scavenger #14</b>
L* (1)	60.94	59.36	58.35	59.68	60.25	61.92
a* (1)	15.95	18.57	17.25	18.00	18.56	17.28
b* (1)	8.94	8.88	7.89	8.66	8.29	8.57
L* (2)	63.26	59.30	59.81	62.16	60.17	61.10
a* (2)	14.45	18.75	16.65	16.65	18.77	17.40
b* (2)	9.16	9.01	7.83	9.10	7.90	8.04
L* (3)	63.92	58.23	59.44	60.73	60.25	61.59
a* (3)	13.91	19.49	17.64	17.44	18.70	16.72
b* (3)	8.63	8.70	8.35	9.13	8.38	7.87
<b>L* AVERAGE</b>	62.71	58.96	59.20	60.86	60.22	61.54
<b>a* AVERAGE</b>	14.77	18.94	17.18	17.36	18.68	17.13
<b>b* AVERAGE</b>	8.91	8.86	8.02	8.96	8.19	8.16
<b>Date Collected</b>	10/26/12 Day		15			
<i>R&amp;D Lab</i>	<b>Control A9</b>	<b>Test F1#14</b>	<b>Control B33</b>	<b>Test F3#14</b>	<b>Test F4#14</b>	<b>Scavenger #13</b>
MOCON - CO2 (%)	19.3	18.5	18.1	17.5	18.5	16.2
MOCON - O2 (%)	0.331	0.306	0.348	0.260	0.260	0.334
	<b>Control A9</b>	<b>Test F1#14</b>	<b>Control B33</b>	<b>Test F3#14</b>	<b>Test F4#14</b>	<b>Scavenger #13</b>
L* (1)	61.83	52.11	57.05	60.96	59.61	57.80
a* (1)	16.39	19.00	19.33	15.12	16.09	18.94
b* (1)	8.02	9.01	8.56	8.73	8.05	8.45
L* (2)	62.68	52.50	59.01	61.42	59.67	57.75
a* (2)	15.86	18.95	17.83	14.97	16.58	18.86
b* (2)	8.11	9.58	7.81	9.07	7.88	8.62
L* (3)	61.94	53.48	58.31	59.90	59.53	57.51
a* (3)	15.89	18.80	17.86	15.34	16.32	18.64
b* (3)	8.07	8.93	7.50	9.17	8.34	8.56
<b>L* AVERAGE</b>	62.15	52.70	58.12	60.76	59.60	57.69
<b>a* AVERAGE</b>	16.05	18.92	18.34	15.14	16.33	18.81
<b>b* AVERAGE</b>	8.07	9.17	7.96	8.99	8.09	8.54

<b>WEEK 3</b>						
<b>Date Collected</b>	10/29/12 Day		18			
<i>R&amp;D Lab</i>	<b>Control A2</b>	<b>Test F1#2</b>	<b>Control B22</b>	<b>Test F3#2</b>	<b>Test F4#2</b>	<b>Scavenger #2</b>
Observations	None					
MOCON - CO2 (%)	18.2	18.2	17.5	18.6	18.0	16.1
MOCON - O2 (%)	0.342	0.496	0.368	0.369	0.348	0.369
L* (1)						
a* (1)						
b* (1)						
L* (2)		Measurements were not retrievable.				
a* (2)						
b* (2)						
L* (3)						
a* (3)						
b* (3)						
<b>L* AVERAGE</b>	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
<b>a* AVERAGE</b>	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
<b>b* AVERAGE</b>	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
<b>Date Collected</b>	10/31/12 Day		20			
<i>POS Building</i>	<b>Cooler A</b>		<b>Cooler B</b>		<b>Cooler C</b>	
MOCON - CO2 (%)	17.7	18.3	17.0	17.4	19.6	16.9
MOCON - O2 (%)	0.355	0.296	0.297	0.304	0.285	0.256
	<b>Control A10</b>	<b>Test F1#13</b>	<b>Control B32</b>	<b>Test F3#13</b>	<b>Test F4#13</b>	<b>Scavenger #12</b>
L* (1)	59.42	59.22	58.00	58.74	58.86	61.05
a* (1)	18.19	16.85	17.81	17.15	17.41	16.11
b* (1)	8.86	8.59	8.47	8.23	8.63	9.39
L* (2)	59.51	59.20	57.65	58.28	58.90	61.65
a* (2)	18.20	16.90	17.90	17.20	17.61	16.48
b* (2)	8.60	8.41	7.89	7.83	8.30	9.31
L* (3)	58.96	59.17	57.99	58.48	58.73	60.44
a* (3)	18.46	16.74	17.62	17.07	17.65	17.50
b* (3)	8.26	8.28	8.33	8.04	7.67	9.53
<b>L* AVERAGE</b>	59.30	59.20	57.88	58.50	58.83	61.05
<b>a* AVERAGE</b>	18.28	16.83	17.78	17.14	17.56	16.70
<b>b* AVERAGE</b>	8.57	8.43	8.23	8.03	8.20	9.41
<b>Date Collected</b>	11/2/12 Day		22			
<i>R&amp;D Lab</i>	<b>Control A11</b>	<b>Test F1#12</b>	<b>Control B31</b>	<b>Test F3#12</b>	<b>Test F4#12</b>	<b>Scavenger #11</b>
Observations		Slight lines of discolor		Slight lines of discolor	Slight lines of discolor	Sachet present
MOCON - CO2 (%)	17.5	16.6	18.6	16.6	17.4	17.9
MOCON - O2 (%)	0.359	0.369	0.296	0.339	0.299	0.367
	<b>Control A11</b>	<b>Test F1#12</b>	<b>Control B31</b>	<b>Test F3#12</b>	<b>Test F4#12</b>	<b>Scavenger #11</b>
L* (1)	60.10	57.01	57.84	60.43	60.26	59.78
a* (1)	18.19	19.28	20.24	16.88	18.79	18.46
b* (1)	9.53	8.34	10.20	7.86	9.19	8.99
L* (2)	60.57	56.37	57.44	59.76	60.11	60.32
a* (2)	17.94	19.93	20.29	17.44	19.13	17.97
b* (2)	8.56	8.05	9.67	7.86	9.67	8.71
L* (3)	59.59	56.55	57.60	59.82	60.42	60.50
a* (3)	18.26	19.35	20.55	17.48	18.59	17.92
b* (3)	8.28	8.06	8.44	8.01	10.21	8.72
<b>L* AVERAGE</b>	60.09	56.64	57.63	60.00	60.26	60.20
<b>a* AVERAGE</b>	18.13	19.52	20.36	17.27	18.84	18.12
<b>b* AVERAGE</b>	8.79	8.15	9.44	7.91	9.69	8.81

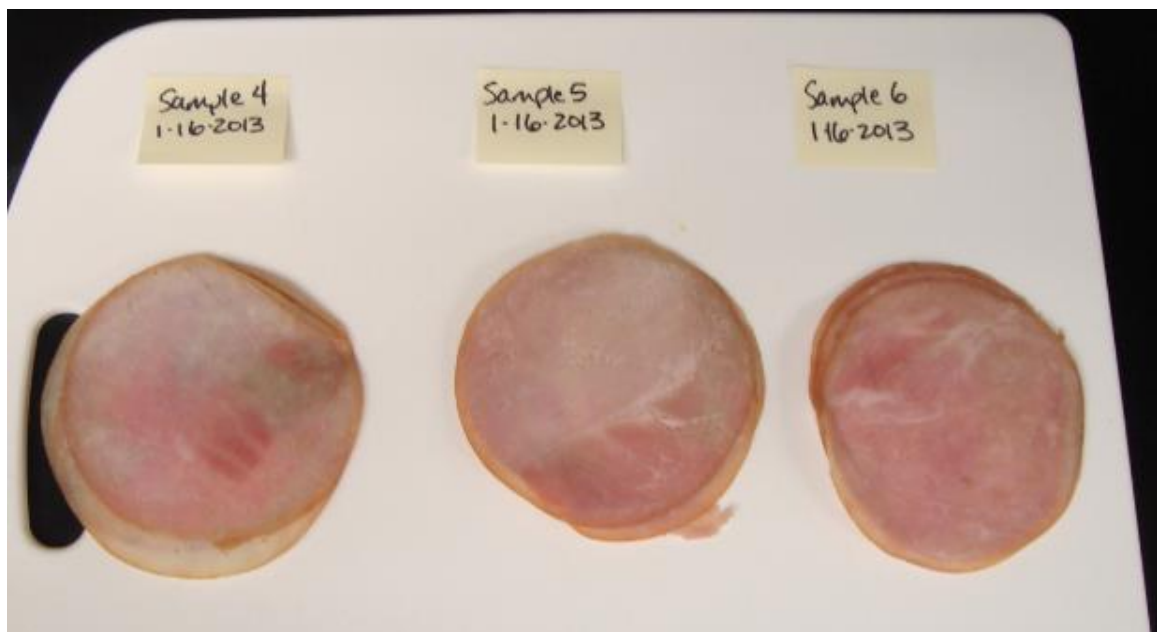
<b>WEEK 4</b>						
<b>Date Collected</b>	<b>11/5/12</b> Day		<b>25</b>			
<i>R&amp;D Lab</i>	<b>Control A12</b>	<b>Test F1#11</b>	<b>Control B30</b>	<b>Test F3#11</b>	<b>Test F4#11</b>	<b>Scavenger #10</b>
Observations						
MOCON - CO2 (%)	16.9	17.4	16.4	17.5	18.4	16.8
MOCON - O2 (%)	0.505	0.368	0.357	0.392	0.392	0.407
	<b>Control A12</b>	<b>Test F1#11</b>	<b>Control B30</b>	<b>Test F3#11</b>	<b>Test F4#11</b>	<b>Scavenger #10</b>
L* (1)	63.27	59.02	61.40	60.79	61.01	59.84
a* (1)	16.18	18.60	17.15	18.25	17.15	18.67
b* (1)	9.34	8.42	8.95	9.16	8.78	8.24
L* (2)	64.04	58.75	63.01	63.20	61.25	59.89
a* (2)	15.95	18.52	16.51	16.59	17.17	18.80
b* (2)	8.71	8.33	8.31	9.25	8.79	8.44
L* (3)	64.28	57.81	62.67	64.03	61.07	58.77
a* (3)	15.29	18.62	16.55	15.29	17.16	19.10
b* (3)	8.70	8.06	8.94	9.36	8.27	8.73
<b>L* AVERAGE</b>	63.86	58.53	62.36	62.67	61.11	59.50
<b>a* AVERAGE</b>	15.81	18.58	16.74	16.71	17.16	18.86
<b>b* AVERAGE</b>	8.92	8.27	8.73	9.26	8.61	8.47
<b>Date Collected</b>	<b>11/7/12</b> Day		<b>27</b>			
MOCON - CO2 (%)	17.4	16.5	16.4	17.6	16.9	19.1
MOCON - O2 (%)	0.308	0.297	0.242	0.345	0.380	0.288
	<b>Control A15</b>	<b>Test F1#10</b>	<b>Control B40</b>	<b>Test F3#10</b>	<b>Test F4#10</b>	<b>Scavenger #20</b>
L* (1)	58.22	59.18	61.65	60.94	58.93	58.78
a* (1)	18.03	18.80	16.40	17.95	18.78	16.74
b* (1)	8.20	8.22	9.28	8.79	8.86	9.80
L* (2)	58.33	59.26	60.65	59.68	59.71	58.96
a* (2)	17.88	18.80	16.93	18.96	18.18	16.97
b* (2)	7.51	8.27	7.99	9.33	8.45	9.20
L* (3)	57.89	59.22	60.17	59.68	59.25	57.72
a* (3)	17.56	18.90	16.54	19.35	18.05	16.97
b* (3)	7.73	8.45	7.90	8.95	8.61	9.30
<b>L* AVERAGE</b>	58.15	59.22	60.82	60.10	59.30	58.49
<b>a* AVERAGE</b>	17.82	18.83	16.62	18.75	18.34	16.89
<b>b* AVERAGE</b>	7.81	8.31	8.39	9.02	8.64	9.43
<b>Date Collected</b>	<b>11/9/12</b> Day		<b>29</b>			
<i>R&amp;D Lab</i>	<b>Control A16</b>	<b>Test F1#19</b>	<b>Control B39</b>	<b>Test F3#19</b>	<b>Test F4#19</b>	<b>Scavenger #19</b>
Observations	Visible lines of discoloration	Very slight discoloration	Visible lines of discoloration	Very slight discoloration		Scavenger present
MOCON - CO2 (%)	17.2	18.0	16.5	17.2	16.8	16.5
MOCON - O2 (%)	0.314	0.337	0.273	0.324	0.340	0.300
	<b>Control A16</b>	<b>Test F1#19</b>	<b>Control B39</b>	<b>Test F3#19</b>	<b>Test F4#19</b>	<b>Scavenger #19</b>
L* (1)	60.90	55.91	57.71	63.34	59.45	57.72
a* (1)	17.84	19.16	17.76	16.77	17.21	18.91
b* (1)	8.19	8.93	9.34	7.29	7.65	8.37
L* (2)	59.12	56.28	57.62	62.76	60.17	57.73
a* (2)	18.36	19.36	17.85	16.96	16.74	18.48
b* (2)	8.99	7.88	7.93	8.82	8.19	8.76
L* (3)	59.63	57.74	57.51	60.70	59.85	56.96
a* (3)	17.66	18.45	17.95	18.11	16.80	19.49
b* (3)	8.89	8.60	8.15	8.98	8.04	9.36
<b>L* AVERAGE</b>	59.88	56.64	57.61	62.27	59.82	57.47
<b>a* AVERAGE</b>	17.95	18.99	17.85	17.28	16.92	18.96
<b>b* AVERAGE</b>	8.69	8.47	8.47	8.36	7.96	8.83
<b>Date Collected</b>	<b>11/12/12</b> Day		<b>32</b>			
<i>R&amp;D Lab</i>	<b>Control A7</b>	<b>Test F1#20</b>	<b>Control B27</b>	<b>Test F3#20</b>	<b>Test F4#20</b>	<b>Scavenger #7</b>
MOCON - CO2 (%)	16.9	16.4	15.1	14.3	15.8	15.3
MOCON - O2 (%)	0.333	0.309	0.304	0.266	0.286	0.320
	<b>Control A7</b>	<b>Test F1#20</b>	<b>Control B27</b>	<b>Test F3#20</b>	<b>Test F4#20</b>	<b>Scavenger #7</b>
L* (1)	60.68	58.41	55.90	57.91	59.05	58.23
a* (1)	14.73	17.20	18.99	17.09	16.67	16.45
b* (1)	9.53	9.55	9.21	10.27	8.92	9.92
L* (2)	60.96	58.46	56.71	57.29	59.14	58.01
a* (2)	14.66	17.05	18.45	17.12	16.91	17.31
b* (2)	9.61	9.40	9.04	8.91	8.43	9.96
L* (3)	60.65	58.43	57.51	57.76	59.66	57.69
a* (3)	15.18	16.72	17.70	16.85	16.71	17.55
b* (3)	9.71	8.68	9.67	8.53	7.53	10.22
<b>L* AVERAGE</b>	60.76	58.43	56.71	57.65	59.28	57.98
<b>a* AVERAGE</b>	14.86	16.99	18.38	17.02	16.76	17.10
<b>b* AVERAGE</b>	9.62	9.21	9.31	9.24	8.29	10.03

## Appendix F – Test 6 Proximity of sandwich to the light source

F.1 Visual appearance of lane A (sample 1), Lane B (sample # 2) and lane C (sample # 3) at day 1. Discoloration is most evident in lane A. Lane A is closest to the light source.



F.2 Visual appearance of lane A (sample 4), Lane B (sample # 5) and lane C (sample # 6) at day 6. Discoloration is more evident on test sample #4 that contained high oxygen (1.56%).



F.3 Visual appearance of lane A (sample 7), Lane B (sample # 8) and lane C (sample # 9) at day 14. Discoloration is evident on all samples. Lane A is closest to the light source.

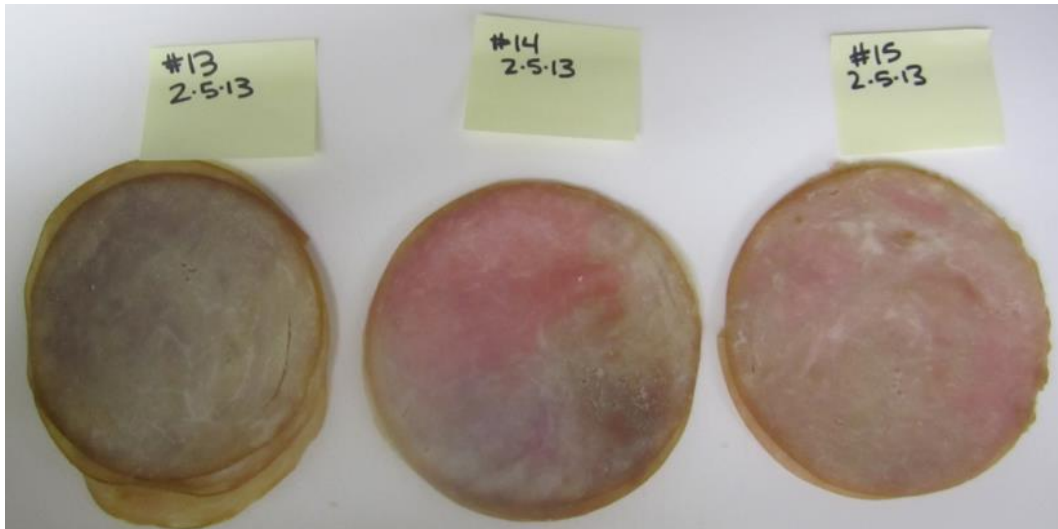


F.4 Visual appearance of lane A (sample 10), Lane B (sample # 11) and lane C (sample # 12) at day 21. Discoloration is evident on all samples, however Lane A visually appears as more discolored than the other lanes. Lane A is closest to the light source.





F.5 Visual appearance of lane A (sample 13), Lane B (sample # 14) and lane C (sample # 15) at day 26. Discoloration is evident on all samples; however lane A again demonstrates a greater amount of discoloration. Lane A is closest to the light source.



F.6 Visual appearance of lane A (sample 16), Lane B (sample # 17) and lane C (sample # 18) at day 32. Discoloration is evident on all samples, but appears less discolored in all lanes compared to prior day evaluations.



F.7  $L^*a^*b^*$  and oxygen and carbon dioxide level raw data from test 6 (3 measurements are combine to be the representative Lab\* score for the sample).

Date Collected	1/11/13	Day	1	Date Collected	1/31/13	Day	21
	Sample 1	Sample 2	Sample 3	R&D Lab	Sample 10	Sample 11	Sample 12
MOCON - CO2 (%)	MOCON was not available.			MOCON - CO2 (%)	18.4	18.8	19.1
MOCON - O2 (%)				MOCON - O2 (%)	0.000	0.000	0.000
L* (1) TOP	60.28	65.00	63.18	L* (1) TOP	60.72	59.22	59.97
a* (1)	14.42	15.15	15.49	a* (1)	8.21	17.98	13.84
b* (1)	11.15	8.66	9.92	b* (1)	9.18	9.75	11.08
L* (2) MID	59.73	65.18	64.35	L* (2) MID	59.06	58.52	62.15
a* (2)	14.55	16.13	15.49	a* (2)	12.98	16.87	12.32
b* (2)	11.01	8.63	10.03	b* (2)	8.83	9.61	11.56
L* (3) END	58.92	63.70	62.05	L* (3) END	59.81	59.51	63.99
a* (3)	14.70	17.15	18.67	a* (3)	12.88	13.91	10.15
b* (3)	10.37	8.87	9.52	b* (3)	7.45	8.32	12.38
L* AVERAGE	59.64	64.63	63.19	L* AVERAGE	59.86	59.08	62.04
a* AVERAGE	14.56	16.14	16.55	a* AVERAGE	11.36	16.25	12.10
b* AVERAGE	10.84	8.72	9.82	b* AVERAGE	8.49	9.23	11.67
Date Collected	1/16/12	Day	6	Date Collected	2/5/13	Day	26
R&D Lab	Sample 4	Sample 5	Sample 6	R&D Lab	Sample 13	Sample 14	Sample 15
MOCON - CO2 (%)	17.2	18.4	18.1	MOCON - CO2 (%)	20.5	20.3	21.2
MOCON - O2 (%)	1.560	0.102	0.060	MOCON - O2 (%)	0.000	0.000	0.000
L* (1) TOP	61.58	66.26	59.66	L* (1) TOP	62.05	58.60	60.48
a* (1)	10.21	8.82	16.61	a* (1)	3.95	18.76	11.70
b* (1)	5.72	10.10	9.55	b* (1)	9.53	10.65	10.96
L* (2) MID	56.70	65.05	59.10	L* (2) MID	67.48	58.32	62.58
a* (2)	16.85	11.52	20.35	a* (2)	3.91	17.81	11.73
b* (2)	7.35	9.99	9.02	b* (2)	9.59	8.57	11.31
L* (3) END	59.05	60.21	59.02	L* (3) END	66.88	58.87	61.04
a* (3)	20.28	17.21	21.10	a* (3)	3.87	10.64	11.71
b* (3)	9.82	9.29	9.05	b* (3)	10.18	5.40	12.19
L* AVERAGE	59.11	63.84	59.26	L* AVERAGE	65.47	58.60	61.37
a* AVERAGE	15.78	12.52	19.35	a* AVERAGE	3.91	15.74	11.71
b* AVERAGE	7.63	9.79	9.21	b* AVERAGE	9.77	8.21	11.49
Date Collected	1/24/13	Day	14	Date Collected	2/11/13	Day	32
R&D Lab	Sample 7	Sample 8	Sample 9	R&D Lab	Sample 16	Sample 17	Sample 18
MOCON - CO2 (%)	20.2	19.8	18.1	MOCON - CO2 (%)	21.3	20.1	20.3
MOCON - O2 (%)	0.000	0.000	0.000	MOCON - O2 (%)	0.000	0.000	0.000
L* (1) TOP	58.91	58.32	59.66	L* (1) TOP	58.04	59.93	57.50
a* (1)	12.70	18.88	15.70	a* (1)	19.54	11.16	13.05
b* (1)	8.24	9.01	9.11	b* (1)	9.99	11.83	10.28
L* (2) MID	59.60	58.63	59.57	L* (2) MID	58.55	59.17	57.32
a* (2)	13.45	17.99	14.72	a* (2)	18.95	10.63	13.48
b* (2)	8.46	9.16	9.86	b* (2)	9.63	11.79	9.20
L* (3) END	59.70	60.08	58.20	L* (3) END	59.09	57.83	57.25
a* (3)	12.33	14.66	14.18	a* (3)	13.22	11.59	15.02
b* (3)	8.56	9.48	10.98	b* (3)	10.18	12.08	8.63
L* AVERAGE	59.40	59.01	59.14	L* AVERAGE	58.56	58.98	57.36
a* AVERAGE	12.83	17.18	14.87	a* AVERAGE	17.24	11.13	13.85
b* AVERAGE	8.42	9.22	9.98	b* AVERAGE	9.93	11.90	9.37

## F.8 Multisorb D-50 cc sachet specification

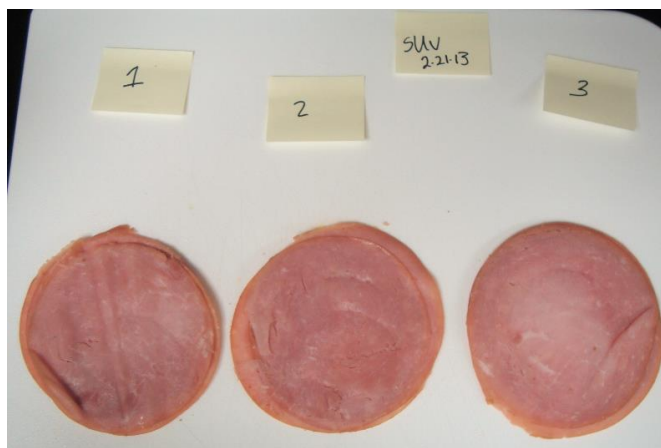
### Multisorb Technologies, Inc. Technical Data Sheet

<b>DATE:</b>	9/28/12
<b>PART NUMBER:</b>	02-02468CG01
<b>PRODUCT NAME:</b>	FreshPax <sup>®</sup> , DFS-50cc, is an oxygen absorbing packet in strip form.
<b>DESCRIPTION:</b>	The DFS-50 oxygen absorbers are designed to absorb a minimum of 50cc of oxygen to modify the atmosphere in a package of dry or semi-moist product with a water activity of 0.3 or more. The rate of absorption is dependent upon the equilibrium relative humidity, temperature, and the composition of the atmosphere within the package.
<b>PHYSICAL ATTRIBUTES:</b>	1.025 wide $\pm$ 0.02 inch X 1.49 long $\pm$ 0.09 inch. (26 wide $\pm$ 0.51mm X 37.8 long $\pm$ 2.3 mm) Holes are punched in the horizontal seal to facilitate use of automatic dispensing and insertion equipment such as APA (Active Pack Automation) and others The DFS-50 is active in air and will begin to react within one-half hour after removal of the protective barrier pouch. It is normally necessary to employ an enclosed unwind chamber with fittings to permit purging with an inert gas, usually nitrogen.
<b>MATERIALS:</b>	The face material is reverse printed, microperforated and bonded to an oil and grease resistant medium with a heat seal layer on the inside.  An Oxygen Indicating tablet in the pouch will be purple in color if oxygen is present in the unopened pouch and pink in the absence of oxygen.
<b>PRINTING:</b>	Red and blue print will include: DO NOT EAT appears on the artwork along with the EU do not eat symbol
<b>PACKAGING:</b>	The product will be as follows: <ul style="list-style-type: none"><li>• 8000 pieces/spool (with 1 oxygen indicating tablet per pouch).</li><li>• 1 spool/case.</li><li>• Total: 8,000 pieces/case.</li><li>• Product label contains following:<ul style="list-style-type: none"><li>- Manufacturer's name</li><li>- Description of product</li><li>- Quantity per container</li><li>- Manufacturer's part number</li><li>- Manufacturer's control number</li></ul></li></ul>
<b>PRODUCT STORAGE:</b>	Cool Dry Location Best if used by date imprinted on label

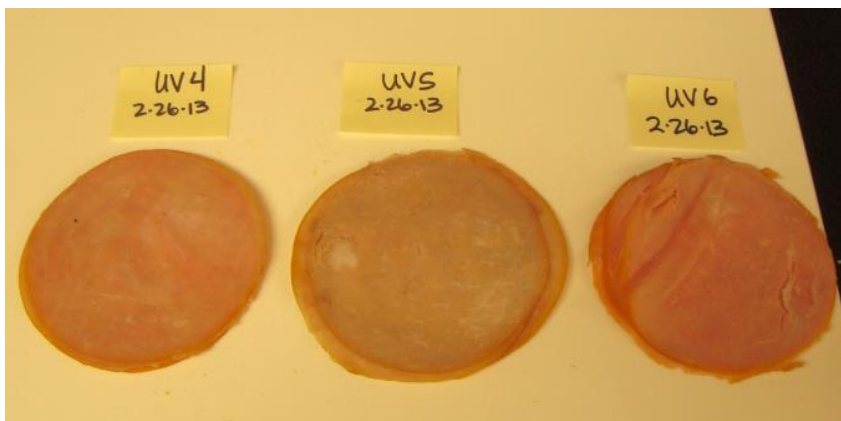
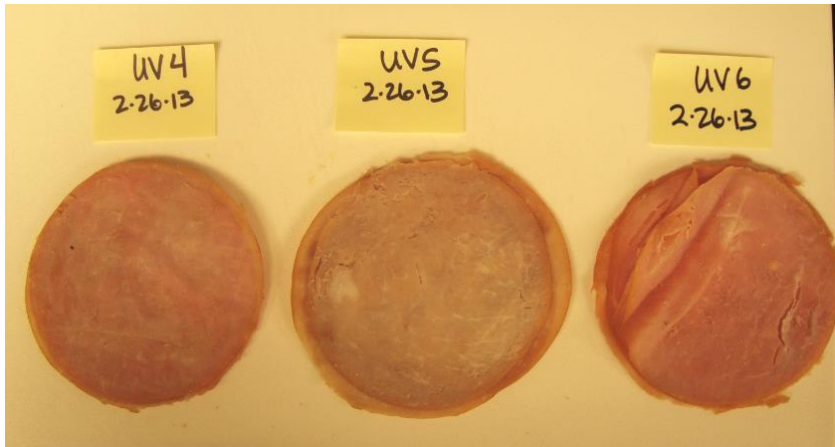
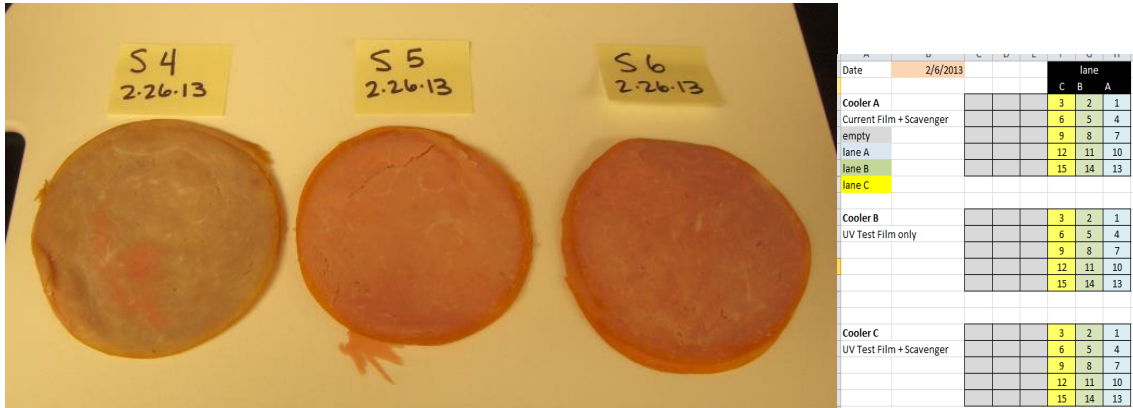
All Statements, technical information and recommendations herein are based on tests we believe to be reliable, but the accuracy and completeness thereof is not guaranteed, and the following is made in lieu of all warranties expressed or implied, including the implied warranties of merchantability and fitness for purpose: Seller's and manufacturer's only obligation shall be to replace such quantity of the product proved to be defective. Before using, user shall determine the suitability of the product for its intended use, and user assumes all risk and liability whatsoever in connection therewith. Neither seller nor manufacturer shall be liable either in tort or in contract for any loss or damage, direct, or incidental, or consequential, arising out of the use of or the inability to use the product. No statement or recommendation not contained herein shall have any force or effect unless in an agreement signed by officers of seller and manufacturer. This information is the property of Multisorb Technologies, Inc. and can only be revised or modified by Multisorb Technologies, Inc.



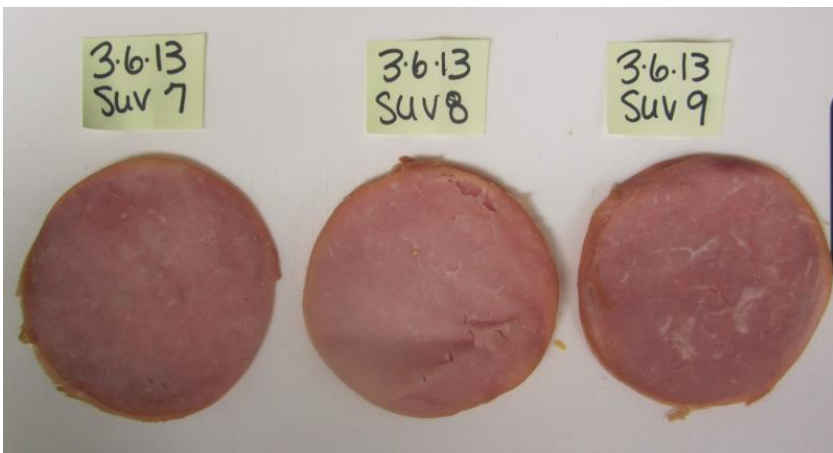
G.1 Visual appearance of lane A (sample 1), Lane B (sample # 2) and lane C (sample # 3) at day 1. Discoloration is not evident in any of the samples. Lane A is closest to the light source.



G.2 Visual appearance of lane A (sample 1), Lane B (sample # 2) and lane C (sample # 3) at day 6. Discoloration is evident in the scavenger treatment which had a higher than average O<sub>2</sub> level at the time of visual inspection (1.52%). Discoloration is also evident in lane B for the other treatments. Lane A is closest to the light source.



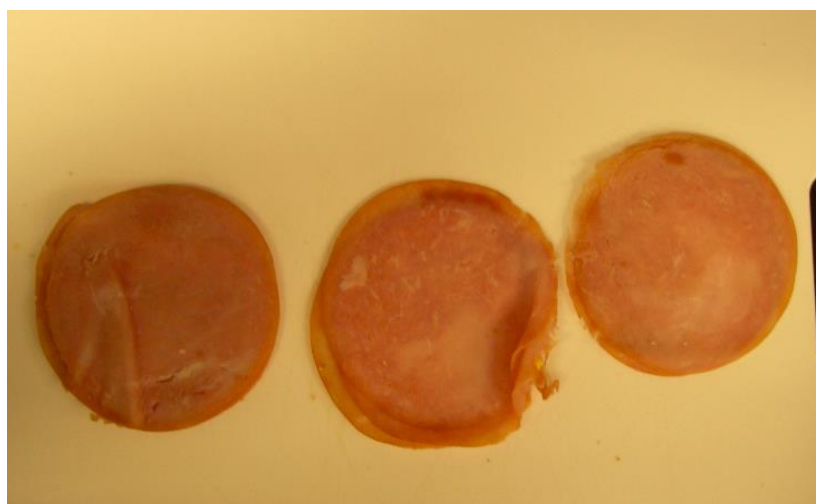
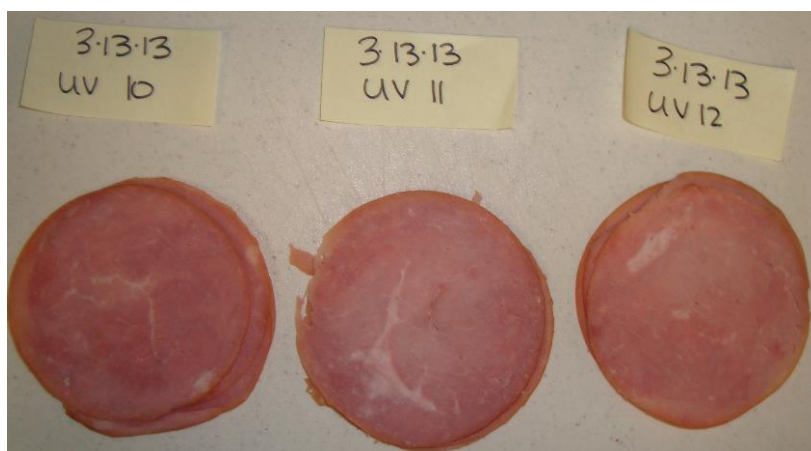
Date					Jane		
	2/6/2013				C	B	A
Cooler A					3	2	1
Current Film + Scavenger					6	5	4
empty					9	8	7
Iane A					12	11	10
Iane B					15	14	13
Iane C							
Cooler B					3	2	1
UV Test Film only					6	5	4
					9	8	7
					12	11	10
					15	14	13
Cooler C					3	2	1
UV Test Film + Scavenger					6	5	4
					9	8	7
					12	11	10
					15	14	13



3.13.13  
S 10

3.13.13  
S 11

3.13.13  
S 12

[illegible]



[illegible]

G.6  $L^*a^*b^*$  raw data (3 measurements are combine to be the representative  $L^*a^*b^*$  score for the sample).

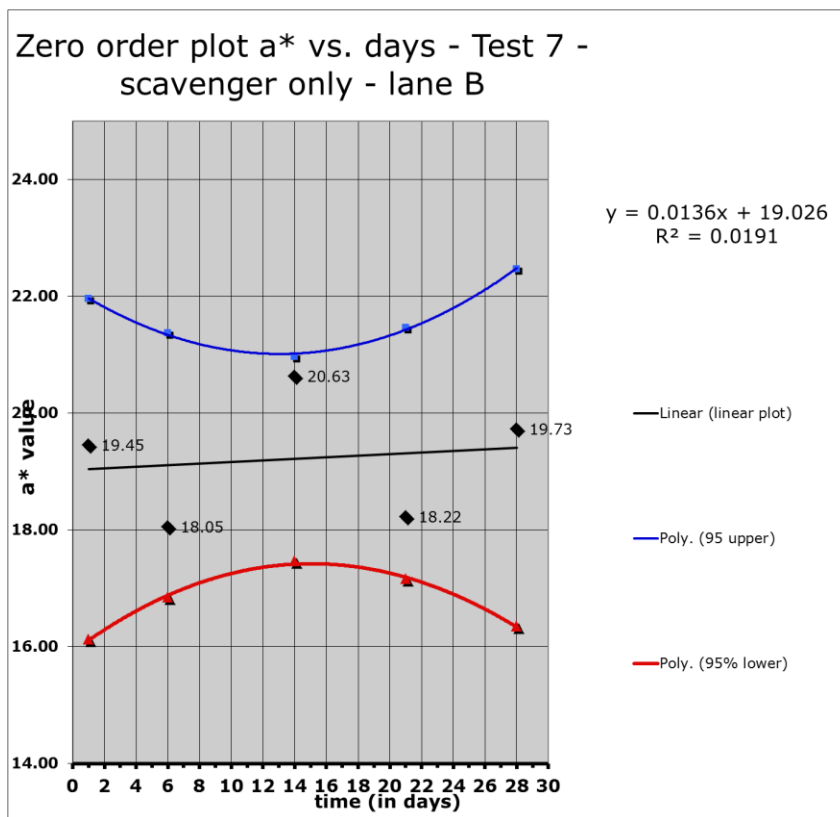
2/21/13	Day	1						
Cooler A			Cooler B			Cooler C		
Sample S #1	Sample S #2	Sample S #3	Sample UV #1	Sample UV #2	Sample UV #3	Sample SUV #1	Sample SUV #2	Sample SUV #3
20.8	20.8	20.2	20.3	20.0	19.6	19.6	20.2	19.0
0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000
63.65	61.89	59.40	63.70	60.14	58.88	59.95	57.69	60.74
17.45	18.37	19.69	17.83	18.62	20.25	20.13	19.88	18.58
8.52	7.97	7.64	9.85	7.75	7.73	7.56	6.62	6.72
58.20	58.04	57.60	61.56	63.07	59.36	61.99	57.58	65.57
21.29	21.29	21.51	18.74	16.87	20.31	18.63	20.63	16.21
7.02	7.02	6.93	9.77	7.93	7.73	7.07	7.41	7.32
61.23	60.66	59.55	60.66	62.39	58.87	58.66	56.76	63.42
18.62	18.69	19.25	19.56	17.70	21.14	19.59	21.31	17.89
7.09	6.90	6.81	9.56	7.86	8.12	6.89	8.08	7.54
61.03	60.20	58.85	61.97	61.87	59.04	60.20	57.34	63.24
19.12	19.45	20.15	18.71	17.73	20.57	19.45	20.61	17.56
7.54	7.30	7.13	9.73	7.85	7.86	7.17	7.37	7.19
	Day	6						
Sample S 4	Sample S 5	Sample S 6	Sample UV 4	Sample UV 5	Sample UV 6	Sample SUV 4	Sample SUV 5	Sample SUV 6
17.3	19.6	19.1	19.6	13.7	20.1	18.9	19.6	19.8
1.52	0.000	0.000	0.000	8.05	0.000	0.000	0.000	0.000
Sample S 4	Sample S 5	Sample S 6	Sample UV 4	Sample UV 5	Sample UV 6	Sample SUV 4	Sample SUV 5	Sample SUV 6
60.45	61.56	58.94	59.84	61.15	59.48	61.40	57.97	66.55
4.98	17.98	19.33	14.04	7.22	18.92	16.73	20.53	17.39
9.21	8.00	8.22	8.86	10.80	8.37	8.59	7.53	10.46
60.01	61.92	61.86	59.85	63.79	58.01	61.38	59.92	66.01
6.86	17.82	19.02	14.01	6.24	20.33	17.72	19.31	17.60
8.81	7.95	7.81	9.30	11.05	7.84	7.59	6.89	9.55
59.01	60.83	61.24	59.22	64.37	56.90	60.96	61.23	63.17
9.47	18.36	19.16	13.63	6.04	21.05	18.34	18.99	18.98
8.21	7.83	7.87	9.67	11.31	8.20	8.35	7.14	9.49
59.82	61.44	60.68	59.64	63.10	58.13	61.25	59.71	65.24
7.10	18.05	19.17	13.89	6.50	20.10	17.60	19.61	17.99
8.74	7.93	7.97	9.28	11.05	8.14	8.18	7.19	9.83
	Day	14						
Sample S #7	Sample S #8	Sample S #9	Sample UV #7	Sample UV #8	Sample UV #9	Sample SUV #7	Sample SUV #8	Sample SUV #9
19.9	19.1	19.0	19.4	19.4	19.6	19.9	19.0	18.2
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
57.57	58.54	58.15	57.11	59.07	59.45	61.38	62.97	58.12
20.31	20.71	21.17	18.86	18.87	18.64	18.54	17.47	19.38
8.14	7.61	8.21	7.97	8.41	8.54	7.71	6.85	6.79
57.55	59.73	59.29	57.06	60.19	60.57	63.56	64.31	58.76
20.74	20.49	20.51	19.93	18.06	18.18	17.95	16.57	19.45
7.08	6.76	7.42	7.72	7.86	8.53	7.54	6.45	6.13
58.72	58.38	58.66	58.86	60.17	60.27	61.97	63.26	58.38
20.88	20.68	20.43	17.34	17.20	18.41	18.59	17.12	19.90
6.95	8.03	7.65	8.30	8.03	8.75	8.04	6.77	6.32
57.95	58.88	58.70	57.68	59.81	60.10	62.30	63.51	58.42
20.64	20.63	20.70	18.71	18.04	18.41	18.36	17.05	19.58
7.39	7.47	7.76	8.00	8.10	8.61	7.76	6.69	6.41

3/13/13 Day		21						
Sample S #10	Sample S #11	Sample S #12	Sample UV #10	Sample UV #11	Sample UV #12	Sample SUV #10	Sample SUV #11	Sample SUV #12
			Noticeable gray				Center is fatty.	
18.4	18.7	19.0	19.5	19.5	19.9	19.5	18.0	18.9
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
56.71	57.15	59.85	57.95	59.55	60.66	57.09	63.13	61.90
20.82	18.84	19.58	16.11	19.37	18.71	18.67	15.07	16.33
7.84	5.82	7.57	10.55	8.89	9.85	6.40	5.33	6.26
56.36	61.56	59.90	58.36	61.31	63.37	58.37	63.86	59.69
21.24	17.55	19.95	15.73	18.58	17.16	18.52	14.55	18.05
7.53	4.96	7.09	9.13	8.49	8.75	5.85	5.50	7.95
56.68	60.32	58.46	58.64	63.05	64.42	56.21	61.85	58.18
20.78	18.28	20.94	14.83	18.14	16.22	19.40	14.95	19.41
8.11	6.21	8.36	9.48	9.16	9.20	7.04	6.00	8.16
56.58	59.68	59.40	58.32	61.30	62.82	57.22	62.95	59.92
20.95	18.22	20.16	15.56	18.70	17.36	18.86	14.86	17.93
7.83	5.66	7.67	9.72	8.85	9.27	6.43	5.61	7.46
3/20/13 Day		28						
Sample S #13	Sample S #14	Sample S #15	Sample UV #13	Sample UV #14	Sample UV #15	Sample SUV #13	Sample SUV #14	Sample SUV #15
18.0	17.5	17.9	19.4	19.1	19.1	18.5	18.1	17.8
0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Sample S #13	Sample S #14	Sample S #15	Sample UV #13	Sample UV #14	Sample UV #15	Sample SUV #13	Sample SUV #14	Sample SUV #15
58.73	60.94	58.43	58.46	57.73	54.34	60.31	56.39	59.42
19.55	18.82	19.71	16.28	19.16	20.41	18.94	21.25	20.21
9.21	8.31	8.25	9.89	8.75	7.26	8.45	8.94	7.81
58.72	58.98	58.87	60.61	58.75	55.28	61.65	58.50	59.82
19.93	20.24	19.46	15.50	18.65	19.21	19.24	20.35	19.92
7.64	7.81	6.86	9.90	9.83	6.10	8.13	7.33	8.01
58.06	59.09	60.42	59.36	57.69	58.18	60.96	57.97	58.91
20.50	20.13	18.44	15.69	18.34	17.98	19.59	20.76	20.24
8.31	8.00	7.65	10.89	10.11	6.88	8.37	6.81	7.97
58.50	59.67	59.24	59.48	58.06	55.93	60.97	57.62	59.38
19.99	19.73	19.20	15.82	18.72	19.20	19.26	20.79	20.12
8.39	8.04	7.59	10.23	9.56	6.75	8.32	7.69	7.93

G.7.a Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the scavenger only sandwiches in Lane B

1. Raw Data:															
# data pairs		Total=	5 This is automatically counted												
Y units		a*		Lane B scavenger only											
X units		days													
STATISTICS															
2. Calculati															
Note after entering Y and X you need to pull down formulas in each column from top to last entry															
r(yi-yes)^2 (xi-xave)^2 xi*yi X^2 y 95%UL y 95%LL Delta predicted average															
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate								
19.45	1.0	378.30	19.45	19.04	1.00	19.45	19.04	0.17	169.00	19.45	1.00	21.96	16.12	5.83	19.04
18.05	6.0	325.92	18.05	19.11	36.00	18.05	19.11	1.11	64.00	108.32	36.00	21.37	16.84	4.53	19.11
20.63	14.0	425.46	20.63	19.22	196.00	20.63	19.22	1.99	0.00	288.77	196.00	20.97	17.46	3.51	19.22
18.22	21.0	332.09	18.22	19.31	441.00	18.22	19.31	1.19	49.00	382.69	441.00	21.47	17.16	4.31	19.31
19.73	28.0	389.27	19.73	19.41	784.00	19.73	19.41	0.10	196.00	552.44	784.00	22.47	16.35	6.12	19.41
Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	yi-yes	(xi-xave)^2	xi*yi	X^2	y 95%UL	y 95%LL	Delta	predicted average

G.7.b Test 7: Scavenger only Ham Zero order plot of  $a^*$  vs. time in lane B (28 days) with 95 % confidence limits calculation





[illegible]

Zero order plot  $a^*$  vs. days - Test 7 - scavenger only lane C

$y = -0.0112x + 20.034$   
 $R^2 = 0.0337$

— Linear (linear plot)

— Poly. (95 upper)

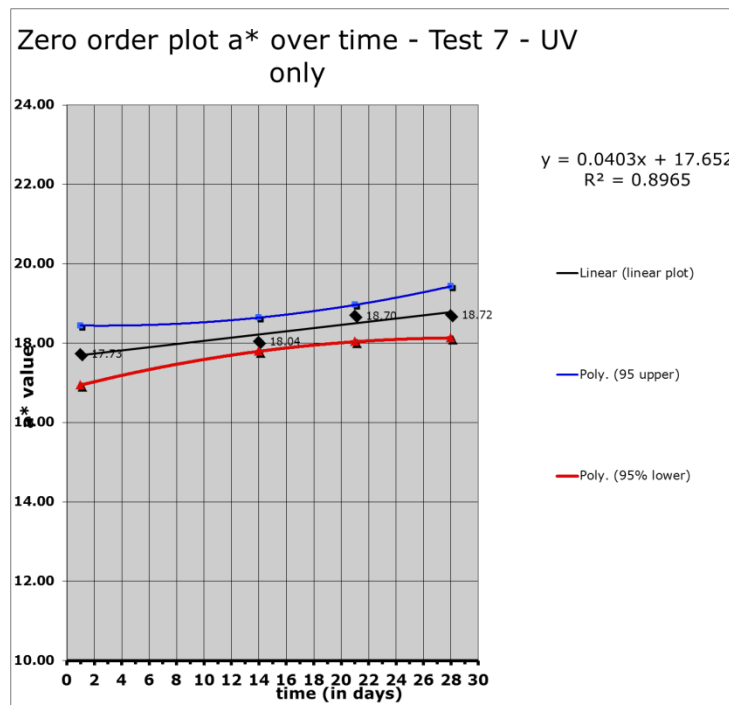
— Poly. (95% lower)

Time (days)	$a^*$ value
1	20.15
6	19.17
14	20.70
21	20.16
28	19.20

G.9.a Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the UV only sandwiches in Lane B

1. Raw Data:																
# data pairs	Total=	4	This is automatically counted													
Y units	a*	Lane B UV only														
X units	days															
STATISTICS																
2. Calculation: Note after entering Y and X you need to pull down formulas in each column from top to last entry (y <sub>i</sub> -y <sub>est</sub> )^2																
	Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(y <sub>i</sub> -y <sub>est</sub> )^2	(x <sub>i</sub> -x <sub>ave</sub> )^2	x <sub>i</sub> *y <sub>i</sub>	X^2	y 95%UL	y 95%LL	Delta	predicted average
	17.73	1.0	314.35	17.73	17.69	1.00	17.73	17.69	0.00	225.00	17.73	1.00	18.44	16.94	1.50	17.69
	18.04	14.0	325.56	18.04	18.22	196.00	18.04	18.22	0.03	4.00	252.61	196.00	18.64	17.79	0.85	18.22
	18.70	21.0	349.57	18.70	18.50	441.00	18.70	18.50	0.04	25.00	392.63	441.00	18.96	18.03	0.93	18.50
	18.72	28.0	350.31	18.72	18.78	784.00	18.72	18.78	0.00	144.00	524.07	784.00	19.43	18.13	1.30	18.78
			0.00	0.00	17.65	0.00	0.00	17.65	311.58	256.00	0.00	0.00	18.44	16.87	1.57	17.65
	Y value	x=time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(y <sub>i</sub> -y <sub>est</sub> )^2	(x <sub>i</sub> -x <sub>ave</sub> )^2	x <sub>i</sub> *Y <sub>i</sub>	X^2	y 95%UL	y 95%LL	Delta	predicted average
	slope=												Standard Error		0.19	
	intercept=												Sum (y <sub>i</sub> -y <sub>est</sub> )		2181.12	
	rsq=												n		4.00	
	± 95% slope												t 95%, 2, n-2=		4.30	
	k upper												x average =		16.00	
	k lower															
	Equations															
	Y = 17.6516      0.0403      * time															
	Sum (x <sub>i</sub> -x <sub>ave</sub> )												2190.00			
	(Sum x <sub>i</sub> )^2												4096.00			
	Sum (y <sub>i</sub> ^2)												1339.79			
	sum y												73.19			
	Sum (x <sub>i</sub> *y <sub>i</sub> )												1187.03			
	sum x												64.00			
	sum (X^2)												1422.00			

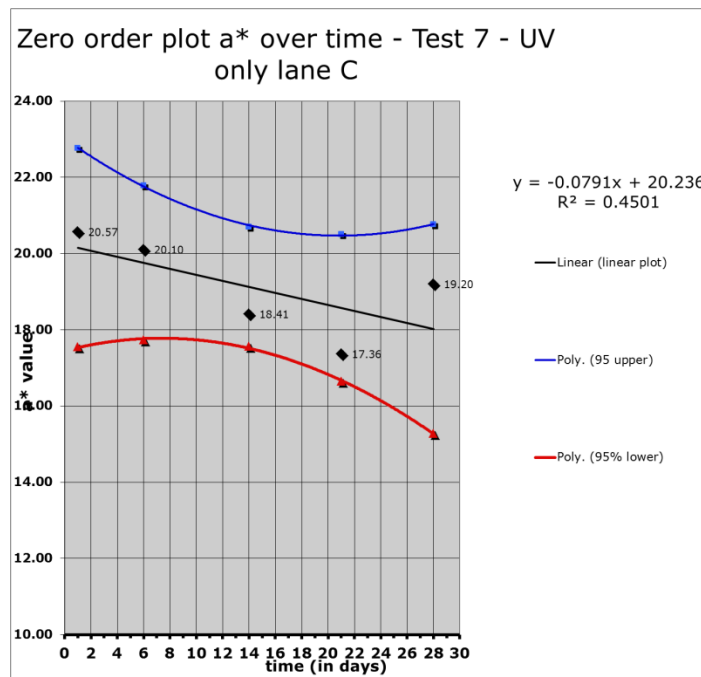
G.9.b Test 7: UV only Ham Zero order plot of  $a^*$  vs. time in lane B (28 days) with 95 % confidence limits calculation



G.10.a Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for the UV only sandwiches in Lane C

1. Raw Data:																
# data pairs Total=			5 This is automatically counted													
Y units			a* Lane C UV only													
X units			days													
STATISTICS																
2. Calculation Note after entering Y and X you need to pull down formulas in each column from top to last entry																
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	
20.57	1.0	422.99	20.57	20.16	1.00	20.57	20.16	0.17	169.00	20.57	1.00	22.77	17.54	5.22	20.16	
20.10	6.0	404.01	20.10	19.76	36.00	20.10	19.76	0.11	64.00	120.60	36.00	21.79	17.73	4.06	19.76	
18.41	14.0	338.93	18.41	19.13	196.00	18.41	19.13	0.52	0.00	257.74	196.00	20.70	17.56	3.14	19.13	
17.36	21.0	301.49	17.36	18.57	441.00	17.36	18.57	1.47	49.00	364.63	441.00	20.51	16.64	3.86	18.57	
19.20	28.0	368.64	19.20	18.02	784.00	19.20	18.02	1.39	196.00	537.60	784.00	20.76	15.28	5.48	18.02	
Y value	x= time	Y^2	Y plot value	Est yi	time^2	yi	yi estimate	(yi-yes)^2	(xi-xave)^2	xi*Yi	X^2	y 95%UL	y 95%LL	Delta	predicted average	
slope=												Standard Error		1.10		
intercept=												Sum (yi-yes)		2460.58		
rsq=												n		5.00		
± 95% slope												t 95%, 2, n-2=		3.18		
k upper												x average =		14.00		
k lower																
Equations																
Y = 20.2358 -0.0791 * time																

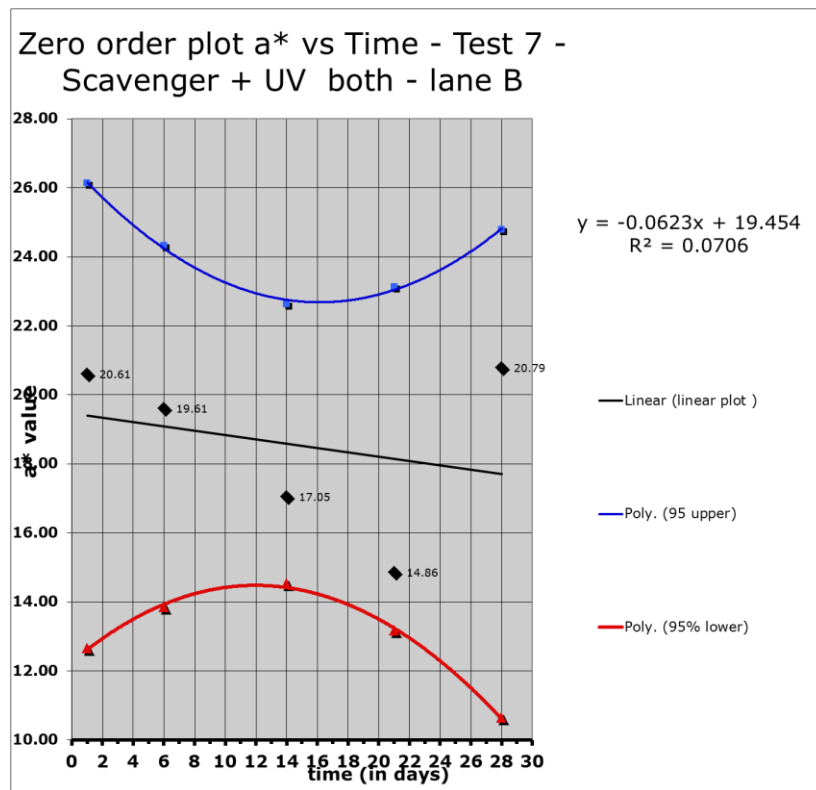
G.10.b Test 7: UV only Ham Zero order plot of  $a^*$  vs. time in lane C (28 days) with 95 % confidence limits calculation



G.11.a Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for combined UV and scavenger sandwiches in Lane B

1. Raw Data:																	
# data pairs	Total=	5	This is automatically counted														
Y units	a*	Lane B - Scavenger + UV															
X units	days																
STATISTICS																	
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry (y <sub>i</sub> -y <sub>est</sub> ) <sup>2</sup>																	
	Y value	x= time	Y <sup>2</sup>	Y plot value	Est y <sub>i</sub>	time <sup>2</sup>	y <sub>i</sub>	y <sub>i</sub> estimate	(y <sub>i</sub> -y <sub>est</sub> ) <sup>2</sup>	(x <sub>i</sub> -x <sub>ave</sub> ) <sup>2</sup>	x <sub>i</sub> *y <sub>i</sub>	X <sup>2</sup>	y 95%UL	y 95%LL	Delta	predicted average	
	20.61	1.0	424.63	20.61	19.39	1.00	20.61	19.39	1.48	169.00	20.61	1.00	26.14	12.65	13.49	19.39	
	19.61	6.0	384.55	19.61	19.08	36.00	19.61	19.08	0.28	64.00	117.66	36.00	24.32	13.84	10.48	19.08	
	17.05	14.0	290.82	17.05	18.58	196.00	17.05	18.58	2.34	0.00	238.75	196.00	22.64	14.53	8.11	18.58	
	14.86	21.0	220.72	14.86	18.15	441.00	14.86	18.15	10.83	49.00	311.99	441.00	23.13	13.16	9.97	18.15	
	20.79	28.0	432.09	20.79	17.71	784.00	20.79	17.71	9.46	196.00	582.03	784.00	24.79	10.63	14.16	17.71	
	Y value	x= time	Y <sup>2</sup>	Y plot value	Est y <sub>i</sub>	time <sup>2</sup>	y <sub>i</sub>	y <sub>i</sub> estimate	(y <sub>i</sub> -y <sub>est</sub> ) <sup>2</sup>	(x <sub>i</sub> -x <sub>ave</sub> ) <sup>2</sup>	x <sub>i</sub> *y <sub>i</sub>	X <sup>2</sup>	y 95%UL	y 95%LL	Delta	predicted average	
	slope=												-0.0623	Standard Error			2.85
	intercept=												19.4542	Sum (y <sub>i</sub> -y <sub>est</sub> )			2295.17
	rsq=												0.0706	n			5.00
	± 95% slope												0.4146	t 95%, 2, n-2=			3.18
	k upper												0.3524	x average =			14.00
	k lower												-0.4769				
	Equations													Sum (x <sub>i</sub> -x <sub>ave</sub> )			1654.00
	Y = 19.4542 -0.0623 * time													(Sum x) <sup>2</sup>			4900.00
														Sum (y <sup>2</sup> )			1752.81
														sum y			92.91
														Sum (x <sub>i</sub> *y <sub>i</sub> )			1271.03
														sum x			70.00
														sum (X <sup>2</sup> )			1458.00

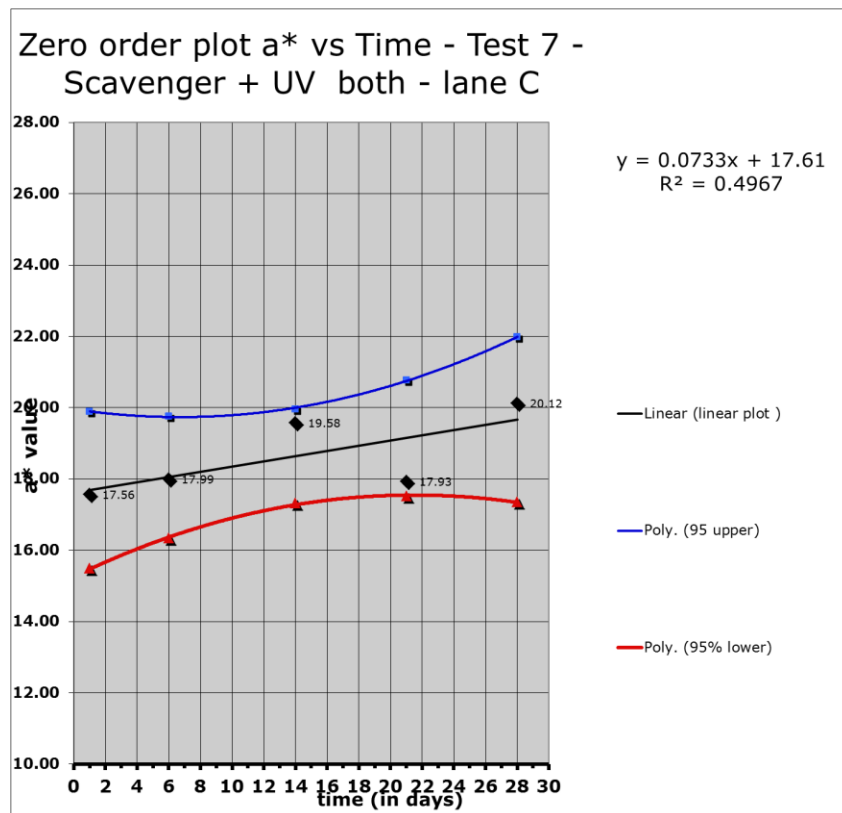
G.11.b Test 7: combined UV and scavenger Ham Zero order plot of  $a^*$  vs. time in lane B (28 days) with 95 % confidence limits calculation



G.12.a Test 7  $a^*$  data input sheet for establishing slope and slope upper and lower value at the 95% CL for combined UV and scavenger sandwiches in Lane C

1. Raw Data:															
# data pairs Total=		5 This is automatically counted													
Y units		a* Lane C - Scavenger + UV													
X units		days													
STATISTICS															
2. Calculations: Note after entering Y and X you need to pull down formulas in each column from top to last entry row (y1-yes)^2															
Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	y1 estimate	(y1-yes)^2	(xi-xave)^2	xi*y1	X^2	y 95%UL	y 95%LL	Delta	predictor average
17.56	1.0	308.35	17.56	17.68	1.00	17.56	17.68	0.02	169.00	17.56	1.00	19.89	15.48	4.41	17.68
17.99	6.0	323.64	17.99	18.05	36.00	17.99	18.05	0.00	64.00	107.94	36.00	19.76	16.34	3.42	18.05
19.58	14.0	383.25	19.58	18.64	196.00	19.58	18.64	0.88	0.00	274.07	196.00	19.96	17.31	2.65	18.64
17.93	21.0	321.48	17.93	19.15	441.00	17.93	19.15	1.49	49.00	376.53	441.00	20.78	17.52	3.26	19.15
20.12	28.0	404.95	20.12	19.66	784.00	20.12	19.66	0.21	196.00	563.45	784.00	21.98	17.35	4.63	19.66
Y value	x= time	Y^2	Y plot value	Est y1	time^2	y1	y1 estimate	(y1-yes)^2	(xi-xave)^2	xi*y1	X^2	y 95%UL	y 95%LL	Delta	predictor average
STATISTICS															
												Standard Error	0.93		
												Sum (y1-yes)	1863.24		
												n	5.00		
												t 95% ,2,n-2=	3.18		
												x average =	14.00		
												Sum (xi-xave)	1654.00		
												(Sum x)^2	4900.00		
												Sum (y^2)	1741.67		
												sum y	93.18		
												Sum (xi*y1)	1339.56		
												sum x	70.00		
												sum (X^2)	1458.00		
Equations															
Y = 17.6098 + 0.0733 * time															
slope= 0.0733															
intercept= 17.6098															
rsq= 0.4967															
± 95% slope= 0.1355															
k upper= 0.2088															
k lower= -0.0622															

G.12.b Test 7: combined UV and scavenger Ham Zero order plot of  $a^*$  vs. time in lane C (28 days) with 95 % confidence limits calculation



## G.13 Multisorb D-50 cc oxygen scavenging sachet specification sheet

### Multisorb Technologies, Inc. Technical Data Sheet

DATE:	9/28/12
PART NUMBER:	02-02468CG01
PRODUCT NAME:	FreshPax <sup>®</sup> , DFS-50cc, is an oxygen absorbing packet in strip form.
DESCRIPTION:	The DFS-50 oxygen absorbers are designed to absorb a minimum of 50cc of oxygen to modify the atmosphere in a package of dry or semi-moist product with a water activity of 0.3 or more. The rate of absorption is dependent upon the equilibrium relative humidity, temperature, and the composition of the atmosphere within the package.
PHYSICAL ATTRIBUTES:	1.025 wide $\pm$ 0.02 inch X 1.49 long $\pm$ 0.09 inch. (26 wide $\pm$ 0.51mm X 37.8 long $\pm$ 2.3 mm) Holes are punched in the horizontal seal to facilitate use of automatic dispensing and insertion equipment such as APA (Active Pack Automation) and others The DFS-50 is active in air and will begin to react within one-half hour after removal of the protective barrier pouch. It is normally necessary to employ an enclosed unwind chamber with fittings to permit purging with an inert gas, usually nitrogen.
MATERIALS:	The face material is reverse printed, microperforated and bonded to an oil and grease resistant medium with a heat seal layer on the inside.  An Oxygen Indicating tablet in the pouch will be purple in color if oxygen is present in the unopened pouch and pink in the absence of oxygen.
PRINTING:	Red and blue print will include: DO NOT EAT appears on the artwork along with the EU do not eat symbol
PACKAGING:	The product will be as follows: <ul style="list-style-type: none"><li>• 8000 pieces/spool (with 1 oxygen indicating tablet per pouch).</li><li>• 1 spool/case.</li><li>• Total: 8,000 pieces/case.</li><li>• Product label contains following:<ul style="list-style-type: none"><li>- Manufacturer's name</li><li>- Description of product</li><li>- Quantity per container</li><li>- Manufacturer's part number</li><li>- Manufacturer's control number</li></ul></li></ul>
PRODUCT STORAGE:	Cool Dry Location Best if used by date imprinted on label

All Statements, technical information and recommendations herein are based on tests we believe to be reliable, but the accuracy and completeness thereof is not guaranteed, and the following is made in lieu of all warranties expressed or implied, including the implied warranties of merchantability and fitness for purpose. Seller's and manufacturer's only obligation shall be to replace such quantity of the product proved to be defective. Before using, user shall determine the suitability of the product for its intended use, and user assumes all risk and liability whatsoever in connection therewith. Neither seller nor manufacturer shall be liable either in tort or in contract for any loss or damage, direct, or incidental, or consequential, arising out of the use of or the inability to use the product. No statement or recommendation not contained herein shall have any force or effect unless in an agreement signed by officers of seller and manufacturer. This information is the property of Multisorb Technologies, Inc. and can only be revised or modified by Multisorb Technologies, Inc.

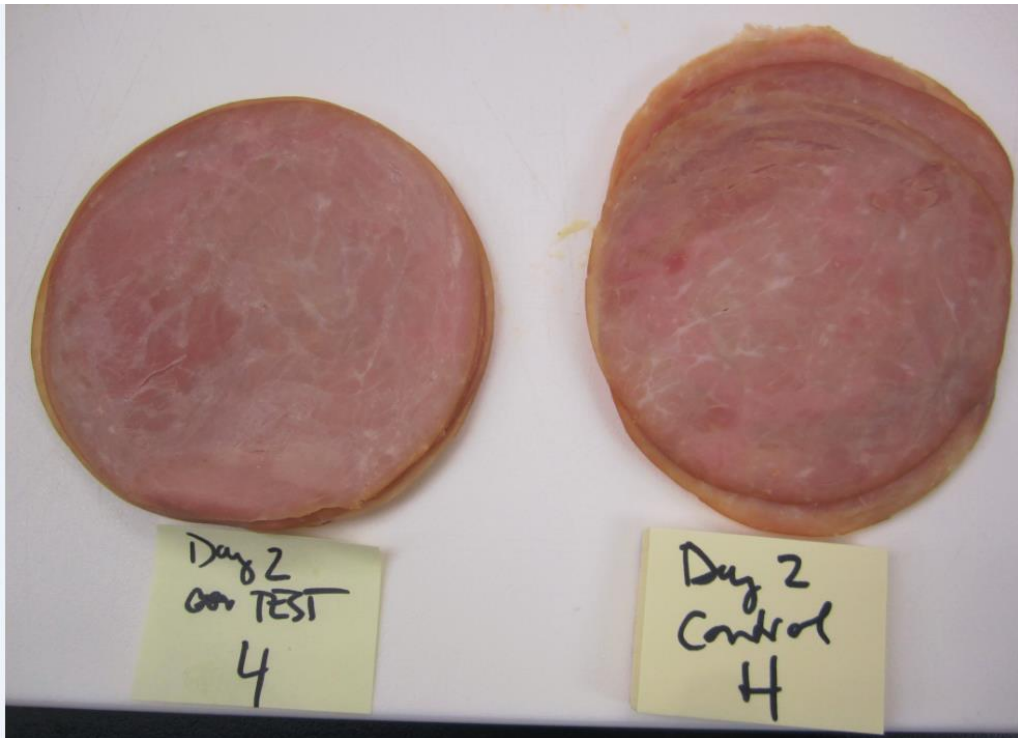
## Appendix H – Test 8 Non-ferrous based oxygen scavenging film

H.1 1) Visual appearance of lane A (sample 1) for test and control out of the package at day 1, 2) Visual appearance of Lane A (sample 1), Lane B (sample # 2) and lane C (sample # 3) at day 1 in the package. No significant discoloration is observed in either application. 3) Cooler set up diagram Lane A is closest to the light source.



Meat Discoloration Study 8			
Cooler A	3	2	1
Control Current Film	6	5	4
	9	8	7
	12	11	10
	15	14	13
	18	17	16
Cooler C	3	2	1
Cryovac Test Film	6	5	4
	9	8	7
	12	11	10
	15	14	13
	18	17	16

H.2 Visual appearance of lane A ham out of the package (test and control sample 4) at day 2. Discoloration is noted in both samples

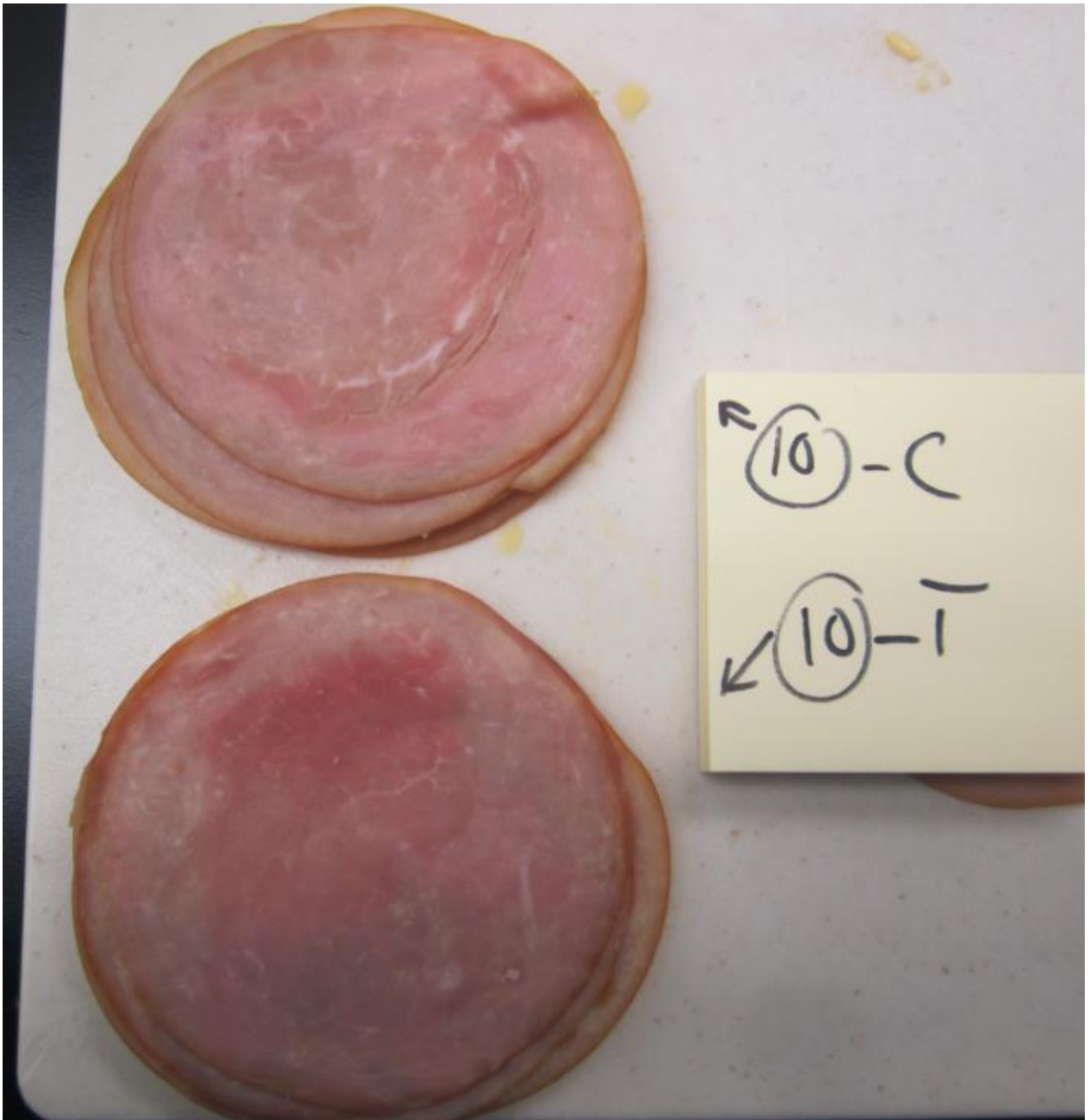


H.3 Visual appearance of lane A (control and test sample 7) at day 3.

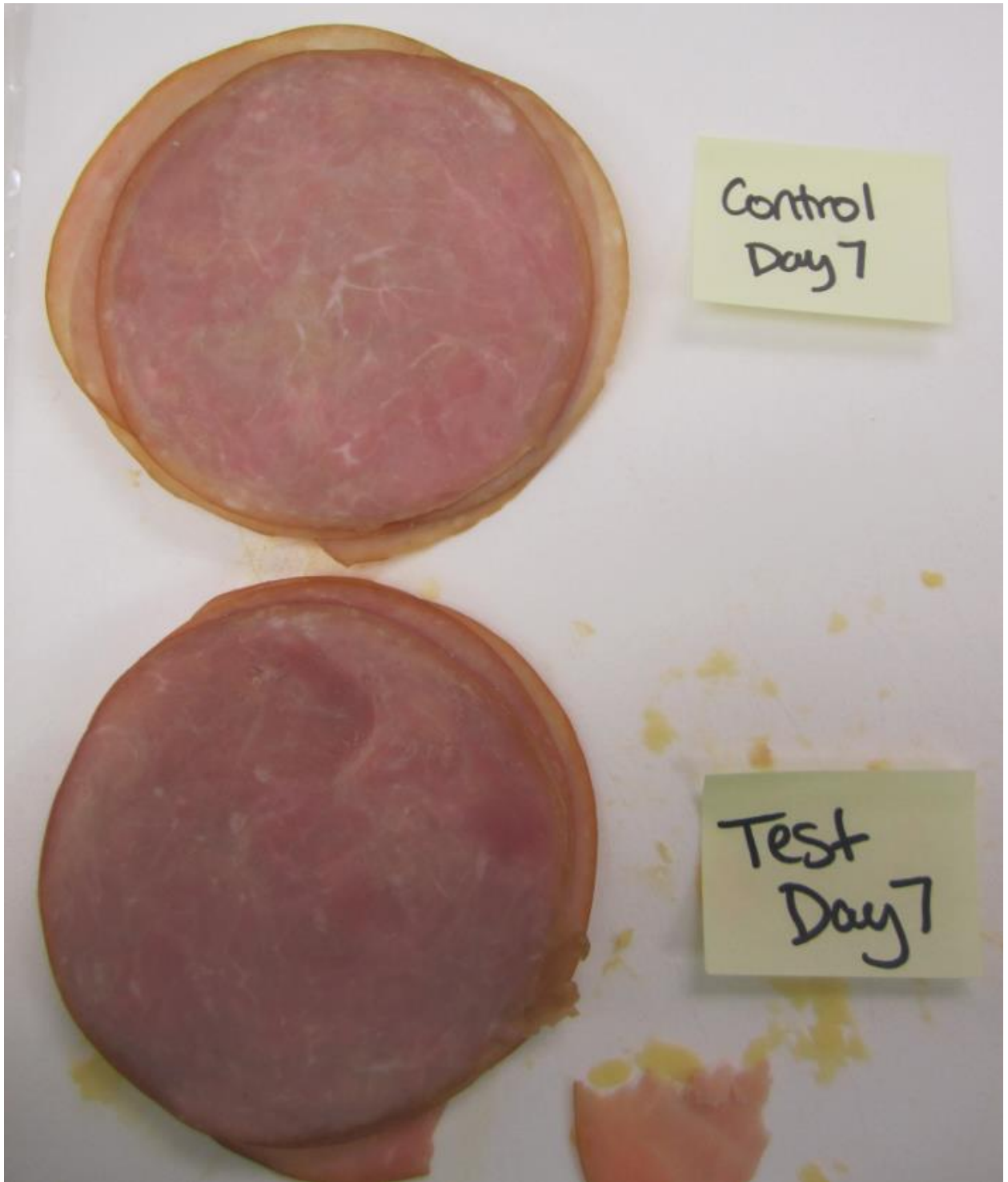




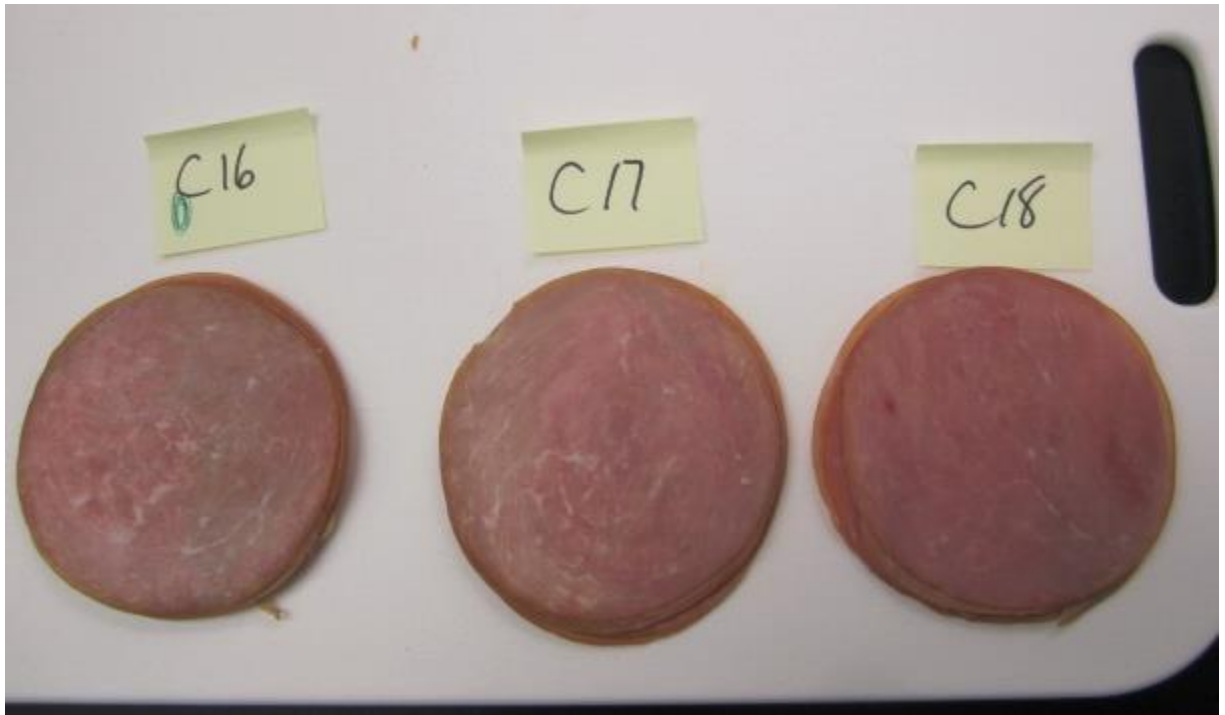
H.4 Visual appearance of lane A product (control and test sample 10) at day 4.  
Discoloration is noted in both treatments



H.5 Visual appearance of lane A ham out of the package (test and control sample 4) at day 7. Discoloration is noted in both samples



H.6 Visual appearance of lane A (sample 16), Lane B (sample # 17) and lane C (sample # 19) at day 30. C = Control, T = non-ferrous scavenging film. Discoloration is evident in both samples.




H.7  $L^*a^*b^*$  raw data test 8 (3 measurements are combine to be the representative  $L^*a^*b^*$  score for the samples). (Carbon Dioxide (CO<sub>2</sub>) and Oxygen (O<sub>2</sub>) raw date included)


				Convert ppm to percentage by dividing by 1		
Date Collected	1/7/14 Day		1			
MOCON	Sample C #1	Sample C #2	Sample C #3	Sample T #1	Sample T #2	Sample T #3
Observations				Very fatty sample		
MOCON - CO2 (%)	18.8	19.2	18.5	19.4	18.8	18.8
MOCON - O2 (%)	0.075	0.172	0.241	0.020	0.070	0.090
Colorimeter	Sample C #1	Sample C #2	Sample C #3	Sample T #1	Sample T #2	Sample T #3
L* (1) TOP	58.29	59.03	58.30	58.88	55.04	57.75
a* (1)	15.36	17.14	17.29	15.97	18.55	18.28
b* (1)	9.99	7.06	6.97	6.09	5.01	6.32
L* (2) MID	59.48	58.27	58.54	61.11	56.94	58.23
a* (2)	15.93	17.18	17.99	15.74	17.88	18.03
b* (2)	8.06	6.60	7.50	7.31	5.06	6.41
L* (3) END	58.78	57.55	57.95	58.16	56.72	57.59
a* (3)	16.16	18.85	18.60	17.20	18.16	18.27
b* (3)	6.71	7.20	7.49	7.66	5.80	6.05
L* AVERAGE	58.85	58.28	58.26	59.38	56.23	57.86
a* AVERAGE	15.82	17.72	17.96	16.30	18.20	18.19
b* AVERAGE	8.25	6.95	7.32	7.02	5.29	6.26
OVERALL AVERAGE L*	58.47			57.82		
OVERALL AVERAGE a*	17.17			17.56		
OVERALL AVERAGE b*	7.51			6.19		
Date Collected	1/8/14 Day		2			
MOCON	Sample C #4	Sample C #5	Sample C #6	Sample T #4	Sample T #5	Sample T #6
Observations	Similar to Test #4			Similar to Control #4		
MOCON - CO2 (%)	17.7	17.2	16.3	16.7	16.3	16.5
MOCON - O2 (%)	0.091	0.053	0.063	0.051	0.132	0.160
Colorimeter	Sample C #4	Sample C #5	Sample C #6	Sample T #4	Sample T #5	Sample T #6
L* (1) TOP	60.40	63.32	58.85	58.08	60.29	58.83
a* (1)	13.57	14.96	17.04	16.99	16.54	17.16
b* (1)	8.96	6.53	7.39	7.91	6.61	5.80
L* (2) MID	60.54	63.50	57.38	60.04	60.83	59.44
a* (2)	13.97	15.32	18.19	13.23	15.49	16.57
b* (2)	9.11	6.23	7.68	8.48	7.41	5.78
L* (3) END	57.49	61.25	56.50	59.86	60.11	59.65
a* (3)	14.88	15.96	18.79	13.11	14.96	16.29
b* (3)	9.82	7.14	7.26	8.02	7.89	5.72
L* AVERAGE	59.48	62.69	57.58	59.33	60.41	59.31
a* AVERAGE	14.14	15.41	18.01	14.44	15.66	16.67
b* AVERAGE	9.30	6.63	7.44	8.14	7.30	5.77

<b>Date Collected</b>	1/9/13 Day		3			
<b>MOCON</b>	Sample C #7	Sample C #8	Sample C #9	Sample T #7	Sample T #8	Sample T #9
<i>Observations</i>		Leaker - looked like it too (discolored)			Leaker - might have been puncture error - sample was still pink	
MOCON - CO2 (%)	20.7	10.5	20.3	19.6	7.4	20.4
MOCON - O2 (%)	0.021	13.500	0.071	0.102	12.100	0.106
<b>Colorimeter</b>	Sample C #7	Sample C #8	Sample C #9	Sample T #7	Sample T #8	Sample T #9
L* (1) <b>TOP</b>	59.54	61.04	57.19	57.54	58.95	58.09
a* (1)	15.46	7.83	17.28	16.31	16.24	16.88
b* (1)	6.95	9.26	8.29	7.36	6.55	5.95
L* (2) <b>MID</b>	57.71	62.83	60.64	61.96	58.37	57.42
a* (2)	17.65	7.35	14.77	11.97	16.38	17.95
b* (2)	6.72	9.29	6.66	8.45	6.18	5.97
L* (3) <b>END</b>	56.32	61.53	60.65	62.72	57.60	57.15
a* (3)	17.77	7.49	15.14	11.29	16.78	17.88
b* (3)	6.59	9.33	6.26	8.65	5.26	5.79
<b>L* AVERAGE</b>	57.86	61.80	59.49	60.74	58.31	57.55
<b>a* AVERAGE</b>	16.96	7.56	15.73	13.19	16.47	17.57
<b>b* AVERAGE</b>	6.75	9.29	7.07	8.15	6.00	5.90
<b>Date Collected</b>	1/10/14 Day		4			
<b>MOCON</b>	Sample C #10	Sample C #11	Sample C #12	Sample T #10	Sample T #11	Sample T #12
<i>Observations</i>	More discoloration than test but composition was more fat and less lean			Less discoloration than control but center was more lean muscle		
MOCON - CO2 (%)	19.7	19.9	20.6	19.8	19.1	20.8
MOCON - O2 (%)	0.002	0.029	0.268	0.037	0.092	0.089
<b>Colorimeter</b>	Sample C #10	Sample C #11	Sample C #12	Sample T #10	Sample T #11	Sample T #12
L* (1) <b>TOP</b>	58.88	57.72	56.54	56.10	56.51	56.96
a* (1)	13.27	15.92	14.91	17.91	17.95	17.18
b* (1)	8.55	9.33	8.39	6.96	6.05	6.83
L* (2) <b>MID</b>	59.09	58.56	56.59	57.31	55.91	57.48
a* (2)	13.30	15.71	15.01	17.23	17.95	16.97
b* (2)	8.96	9.21	8.10	6.11	5.61	6.69
L* (3) <b>END</b>	58.67	57.90	57.66	58.88	57.65	57.90
a* (3)	14.74	16.64	14.50	15.64	16.89	17.22
b* (3)	8.62	8.95	7.85	6.21	5.65	6.22
<b>L* AVERAGE</b>	58.88	58.06	56.93	57.43	56.69	57.45
<b>a* AVERAGE</b>	13.77	16.09	14.81	16.93	17.60	17.12
<b>b* AVERAGE</b>	8.71	9.16	8.11	6.43	5.77	6.58

<b>Date Collected</b>	1/13/14 Day		7			
<b>MOCON</b>	Sample C #13	Sample C #14	Sample C #15	Sample T #13	Sample T #14	Sample T #15
<i>Observations</i>						Very brown color - Leaker
MOCON - CO2 (%)	21.7	20.4	20.2	20.8	20.6	0.4
MOCON - O2 (%)	0.000	0.006	0.024	0.000	0.038	19.100
<b>Colorimeter</b>	Sample C #13	Sample C #14	Sample C #15	Sample T #13	Sample T #14	Sample T #15
L* (1) <b>TOP</b>	58.87	65.39	60.52	58.56	60.96	59.91
a* (1)	13.87	11.99	15.87	15.43	16.09	8.73
b* (1)	8.63	8.87	6.37	6.83	7.15	10.09
L* (2) <b>MID</b>	60.35	64.52	58.82	58.27	60.02	59.26
a* (2)	13.00	12.74	16.96	16.00	16.72	8.94
b* (2)	8.15	8.00	6.74	6.09	6.87	9.84
L* (3) <b>END</b>	59.15	63.36	57.83	58.25	58.56	60.05
a* (3)	15.06	13.07	17.16	15.66	17.59	8.21
b* (3)	7.53	7.93	7.37	6.04	6.68	9.21
<b>L* AVERAGE</b>	59.46	64.42	59.06	58.36	59.85	59.74
<b>a* AVERAGE</b>	13.98	12.60	16.66	15.70	16.80	8.63
<b>b* AVERAGE</b>	8.10	8.27	6.83	6.32	6.90	9.71
<b>Date Collected</b>	2/5/13 Day		30			
<b>MOCON</b>	Sample C #16	Sample C #17	Sample C #18	Sample T #16	Sample T #17	Sample T #18
<i>Observations</i>						
MOCON - CO2 (%)	20.3	20.6	21.1	22.1	19.7	19.9
MOCON - O2 (%)	0.000	0.000	0.000	0.000	0.000	0.000
<b>Colorimeter</b>	Sample C #_	Sample C #_	Sample C #_	Sample T #_	Sample T #_	Sample T #_
L* (1) <b>TOP</b>	58.95	60.80	58.48	63.11	59.19	60.34
a* (1)	13.84	16.48	18.51	9.90	14.27	16.44
b* (1)	8.91	6.74	6.70	9.00	8.47	6.87
L* (2) <b>MID</b>	59.06	60.94	58.33	61.93	59.54	57.13
a* (2)	13.94	16.37	18.46	10.68	14.70	17.82
b* (2)	8.54	6.44	6.20	8.87	7.74	7.89
L* (3) <b>END</b>	60.53	61.27	58.68	61.32	58.84	57.61
a* (3)	14.82	15.90	18.58	11.50	15.54	17.73
b* (3)	8.00	7.33	5.88	8.35	6.96	8.73
<b>L* AVERAGE</b>	59.51	61.00	58.50	62.12	59.19	58.36
<b>a* AVERAGE</b>	14.20	16.25	18.52	10.69	14.84	17.33
<b>b* AVERAGE</b>	8.48	6.84	6.26	8.74	7.72	7.83

**CRYOVAC**  
Sealed Air Corporation

Active Barrier Films

**Sealed Air**  
**CRYOVAC®**  
Food Packaging Solutions

## Active Barrier Forming Films

Active oxygen barrier, multi-layer coextruded films designed specifically for thermoform and form-fill-seal packaging applications. One of the state-of-the-art Freshness Plus® packaging technologies.

### Film Highlights

- Active oxygen barrier
- Clear coextruded film
- Superior protection against oxidation
- Ideal for oxygen sensitive products and products with a long shelf life
- No effect on metal detection systems

The Active Barrier Films utilize polymer based oxygen scavenging material in multi-layer coextruded films. This new generation resin gives the film superior oxygen protection to reduce or eliminate negative product oxidation. The film can be handled the same as other packaging materials because the controlled scavenging is ready for use without an activation step. Scavenger traps oxygen that diffuses into the film thus providing an additional layer of oxygen protection.

The oxygen and moisture barrier layers of these materials assure increased protection of flavor, color and inhibit rancidity. The oxygen scavenging polymer is invisible to metal detectors allowing processors to use metal detection systems after the packaging operation.

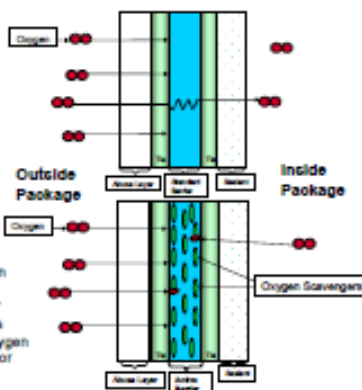


## PROPERTIES

Appearance	Clear	Clear
Forms Available	Single Wound	Single Wound
Thickness Available/mils	5.0	7.0
Tensile Strength/psi @ 73°F.	5170-5860	5040-5710
Elongation/% @ break @ 73°F.	390-410	400-430
Impact Impact/J, energy to break.	0.94	1.44
Tear Resistance/gms	1750	2630
Young's Modulus/psi @ 73°F.	109,000-115,000	105,000 - 107,000
Coefficient of Friction/sealant/stainless steel	Static: 0.529	Static: 0.594
Water Vapor Transmission gm/(24hrs. 100 sq. in.) @ 100% RH	0.2	0.2
<b>Oxygen Permeability/ cc STP/(24 hrs., m<sup>2</sup> atm) @ 73°F., 0% RH</b>	<b>&lt; 1.0*</b>	<b>&lt; 1.0*</b>
Heat Sealing Range/ Degrees C., Thermal	110-180°	110-180°

### Passive Barrier

Standard oxygen barrier films only contain a passive barrier or road block against oxygen



### Active Barrier

Active barrier films contain oxygen scavenging components in the barrier layer. These components actively scavenge any oxygen migrating from the inside or outside of the package

\* Scavenging polymer eliminates oxygen transmission until capacity is exhausted then passive barrier provides an OTR of < 1.0

This information represents our best judgment based on the work done, but the Company assumes no liability whatsoever in connection with the use of information or findings contained herein.

Films comply with the requirements of the Federal Food, Drug and Cosmetics Act, as amended, for the packaging of all foods, with the exception of high alcoholic substances, at temperatures of 100°C and below.

**Sealed Air**  
**CRYOVAC®**  
Food Packaging Solutions





## Cryovac® Oxygen Scavenging Films

The Cryovac Freshness Plus® product line offers state-of-the-art Oxygen Scavenging materials which provide superior protection from oxygen.

Cryovac® oxygen scavenging films remove residual oxygen from your package and then keep oxygen out. This enables food manufacturers to deliver fresher products, over longer periods of time.

OS2030 films remove oxygen from packages, helping to maintain product freshness, color, flavor and nutritional content. The packaging technology helps reduce the growth of aerobic spoilage microorganisms and allows processors to reduce or eliminate preservatives.

The OS films are multilayer coextruded films that incorporate both oxygen barrier and oxygen scavenging layers. The films come with a biaxially oriented PET outer layer and can be trap printed or plain.

Based on a system of proprietary technologies, this polymer-based method reduces oxygen levels in MAP applications. Scavenging begins when a patented UV light triggering unit, installed on the packaging line, activates the film. The scavenging polymer is incorporated into the package and is invisible to the consumer.

These materials do not require moisture to activate the scavenging reaction and are equally effective with wet or dry products. The scavenging polymer is invisible to metal detectors allowing processors to use metal detection systems after the packaging operation.

### Film Highlights

Maximum oxygen protection for flexible packaging

Invisible scavenger in the film rapidly removes headspace oxygen

Scavenger is activated by the customer, on demand

No effect on metal detection systems

# PROPERTIES

Appearance	Clear
Forms Available	Single Wound
Thickness Available/mils	3.1
Tensile Strength/psi @ 73°F.	4740 - 7850
Elongation/% @ break @ 73°F.	70 - 140
Impact Strength/instrumented impact, N.	104
Tear Propagation/gms	49 - 67
Coefficient of Friction	Static: 0.53 Kinetic: 0.39
Water Vapor Transmission gm/(24hrs. 100 sq. in.) @100% RH	8
Passive Oxygen Permeability/ cc STP/(24 hrs., m <sup>2</sup> atm)@ 73°F., 0% RH	2
Heat Sealing Range/ Degrees C., Thermal	110 -180°C
Nominal Seal Strength/ Degrees C., Thermal	8 lbs / linear inch

This information represents our best judgment based on the work done, but the Company assumes no liability whatsoever in connection with the use of information or findings contained herein.

Film complies with the requirements of the Federal Food, Drug and Cosmetics Act, as amended, for the packaging of all foods, with the exception of high alcoholic substances, at temperatures of 65°C and below.

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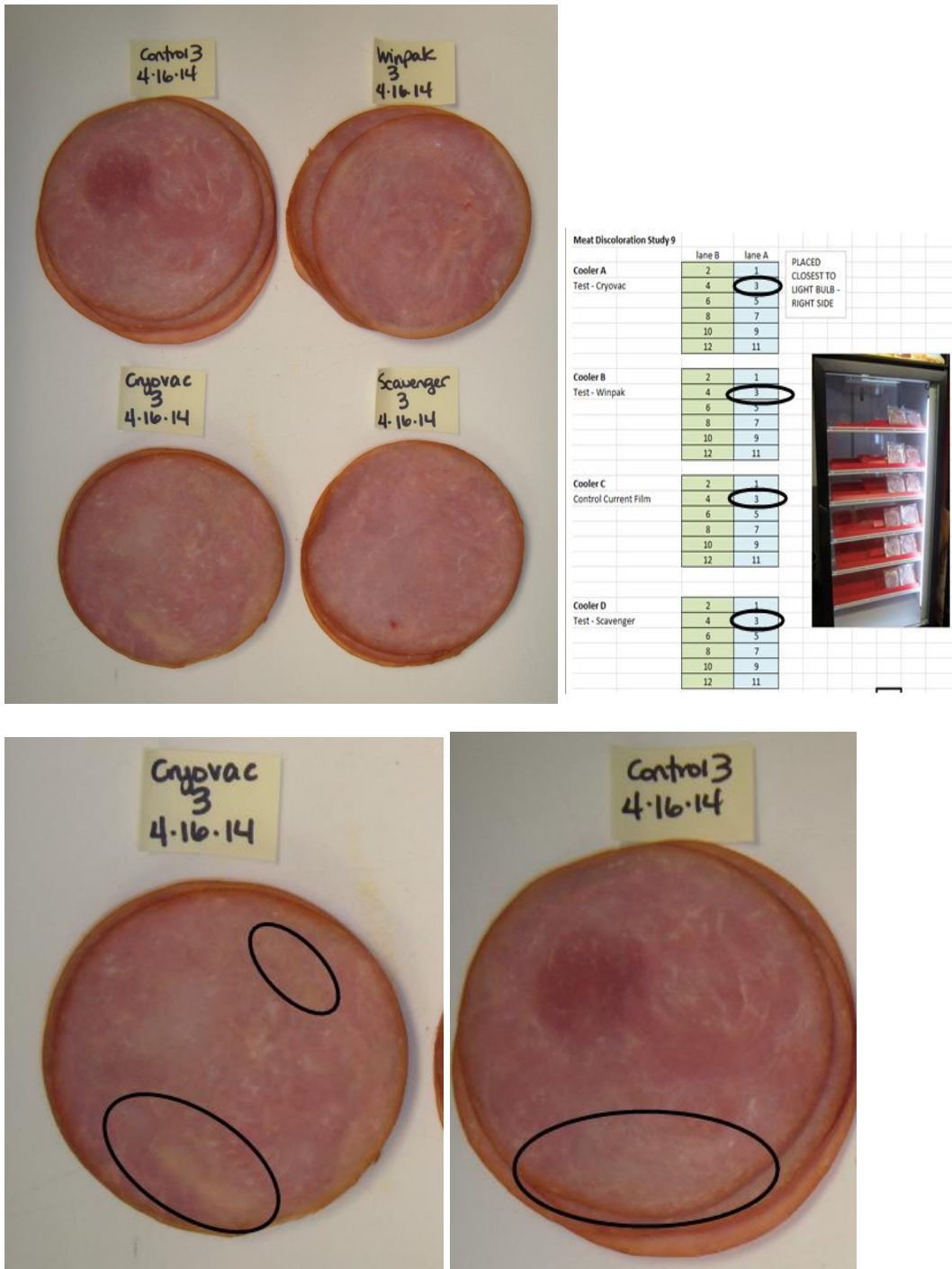
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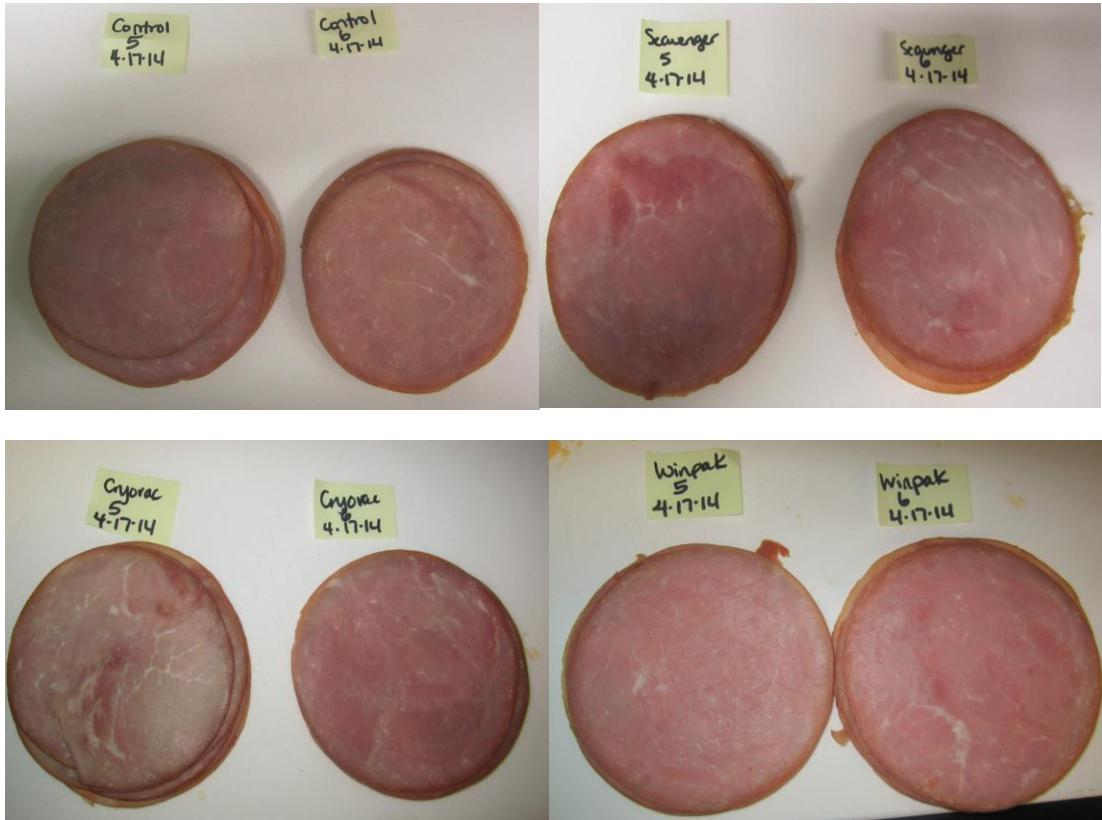
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## Appendix I – Test 9 ferrous based oxygen scavenging film, revisit oxygen scavenging sachet and non-ferrous based scavenging film

I.1 Visual appearance of lane A Control sample 3, Winpak sample # 3, Cryovac sample # 3, and Multisorb sample # 3, at day 2. Discoloration is starting to develop on the control and Cryovac product.



I.2 Visual appearance of lane A & B Control sample 5 & 6, Winpak sample 5 & 6, Cryovac sample 5 & 6, and Multisorb sample 5 & 6, at day 3. Discoloration is more evident on the control and Cryovac product. Lane A discoloration is more evident than lane B

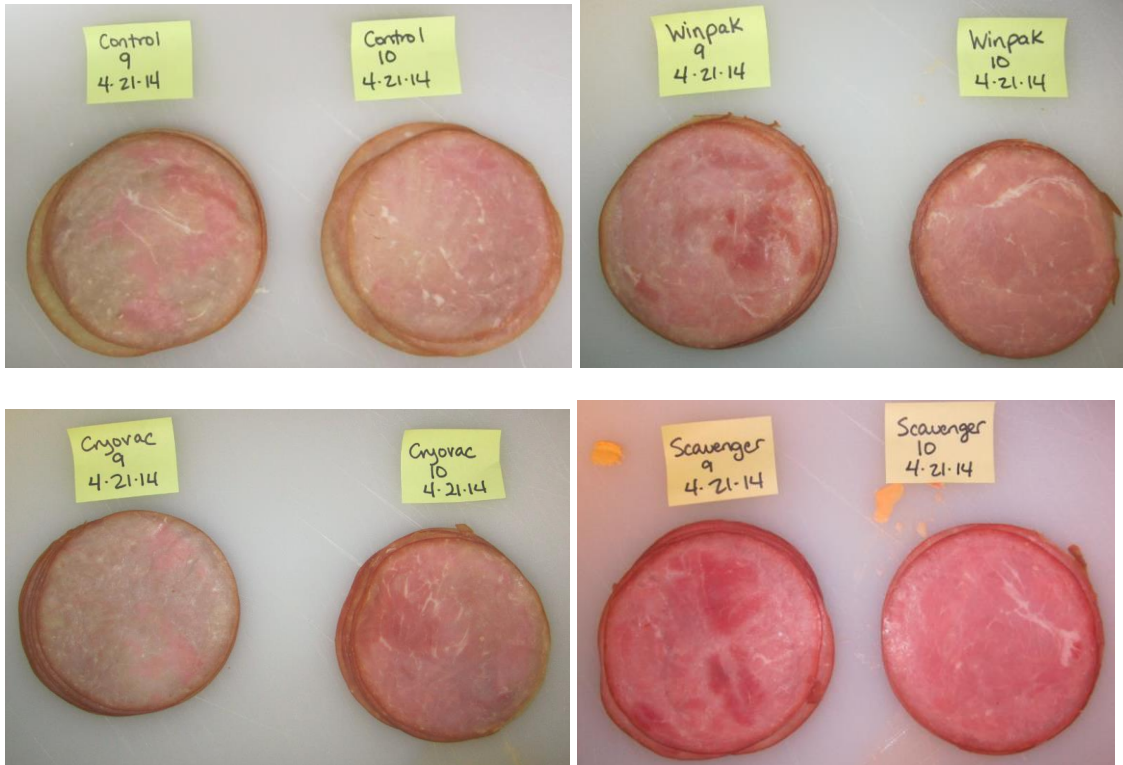


I.3 Visual appearance of lane A Control sample # 7, Winkpak sample # 7, Cryovac sample # 7, and Multisorb sample # 7 at day 4. Visual discoloration is present in all applications except the oxygen scavenging sachet (Multisorb).





I.4 Visual appearance of lane A & B Control sample 9 & 10, Winkpak sample 9 & 10, Cryovac sample 9 & 10, and Multisorb sample 9 & 10, at day 7. Discoloration is more evident on the control and Cryovac product. The Multisorb samples do not show signs of discoloration. Lane A discoloration is more evident than lane B for applications with visual discoloration.



I.5 Visual appearance of lane A Control sample # 11, Winkpak sample # 11, Cryovac sample # 11, and Multisorb sample # 11 at day 30. Visual discoloration is most evident in the control. Similar to previous studies, the visual appearance on several applications have improved in visual appearance.



I.6  $L^*a^*b^*$  raw data (3 measurements are combine to be the representative  $L^*a^*b^*$  score for the sample).

Date Collected	4/15/14 Day		1					
MOCON	Control 1C	Control 2C	Winpak 1	Winpak 2	Cryovac 1	Cryovac 2	Scavenger 1	Scavenger 2
Observations						Leaker - Bread in the seal		
MOCON - CO2 (%)	17.6	17.7	17.1	17.6	17.9	1.0	16.2	16.4
MOCON - O2 (%)	0.249	0.419	0.000	0.038	0.095	18.400	0.000	0.000
Colorimeter	Control 1C	Control 2C	Winpak 1	Winpak 2	Cryovac 1	Cryovac 2	Scavenger 1	Scavenger 2
L* (1) TOP	57.71	52.76	60.10	54.68	56.99	58.01	56.45	57.84
a* (1)	16.07	19.11	16.47	19.95	17.29	12.19	18.58	17.24
b* (1)	7.22	5.85	6.32	6.42	6.98	7.75	4.90	4.85
L* (2) MID	57.30	53.56	57.26	53.68	59.17	57.33	56.80	57.69
a* (2)	17.16	18.78	17.81	20.59	15.58	12.88	18.58	17.93
b* (2)	6.40	5.08	6.23	6.21	6.48	7.69	5.41	5.27
L* (3) END	55.59	53.87	56.24	54.56	58.64	56.98	57.58	56.80
a* (3)	18.52	18.62	18.09	20.12	15.79	12.89	18.00	18.66
b* (3)	5.80	4.87	6.49	6.58	6.79	8.29	6.16	6.25
L* AVERAGE	56.87	53.40	57.87	54.31	58.27	57.44	56.94	57.44
a* AVERAGE	17.25	18.84	17.46	20.22	16.22	12.65	18.39	17.94
b* AVERAGE	6.47	5.27	6.35	6.40	6.75	7.91	5.49	5.46
Date Collected	4/16/14 Day		2					
MOCON	Control 3C	Control 4C	Winpak 3	Winpak 4	Cryovac 3	Cryovac 4	Scavenger 3	Scavenger 4
Observations								
MOCON - CO2 (%)	17.4	17.3	16.2	16.5	16.9	17.4	16.6	17.3
MOCON - O2 (%)	0.262	0.146	0.001	0.000	0.091	0.139	0.000	0.000
Colorimeter	Control 3C	Control 4C	Winpak 3	Winpak 4	Cryovac 3	Cryovac 4	Scavenger 3	Scavenger 4
L* (1) TOP	54.33	60.09	57.11	63.26	58.06	55.71	58.18	59.62
a* (1)	18.43	16.07	18.02	14.08	16.07	16.49	16.65	16.74
b* (1)	6.47	6.55	6.29	6.25	6.93	7.17	5.53	5.40
L* (2) MID	55.09	58.63	57.96	62.24	59.89	54.66	59.29	60.60
a* (2)	17.95	17.17	17.40	14.76	14.23	16.88	15.91	16.17
b* (2)	5.18	6.03	5.43	6.10	6.72	7.70	4.54	6.16
L* (3) END	58.12	58.11	58.86	61.37	60.11	55.94	60.45	61.32
a* (3)	15.81	17.17	16.58	15.53	13.91	16.28	14.99	15.82
b* (3)	5.38	6.28	4.79	6.29	7.11	7.98	4.20	6.79
L* AVERAGE	55.85	58.94	57.98	62.29	59.35	55.44	59.31	60.51
a* AVERAGE	17.40	16.80	17.33	14.79	14.74	16.55	15.85	16.24
b* AVERAGE	5.68	6.29	5.50	6.21	6.92	7.62	4.76	6.12
Date Collected	4/17/14 Day		3					
MOCON	Control 5C	Control 6C	Winpak 5	Winpak 6	Cryovac 5	Cryovac 6	Scavenger 5	Scavenger 6
Observations								
MOCON - CO2 (%)	17.3	18.8	18.0	17.2	18.4	16.1	17.0	16.7
MOCON - O2 (%)	0.283	0.273	0.015	0.120	0.119	0.227	0.093	0.094
Colorimeter	Control 5C	Control 6C	Winpak 5	Winpak 6	Cryovac 5	Cryovac 6	Scavenger 5	Scavenger 6
L* (1) TOP	59.55	58.60	57.19	57.36	61.19	57.72	54.66	62.75
a* (1)	15.16	15.05	17.05	17.69	13.06	16.74	19.63	13.75
b* (1)	6.90	8.12	6.24	5.40	8.61	5.51	5.53	5.42
L* (2) MID	60.12	60.87	57.65	57.60	61.56	58.42	57.69	61.64
a* (2)	14.93	14.01	16.40	17.37	12.18	16.85	17.53	14.55
b* (2)	6.78	8.12	5.62	4.92	8.20	5.28	4.43	4.83
L* (3) END	59.93	59.96	57.54	57.72	61.40	57.32	56.70	58.92
a* (3)	15.94	14.96	16.41	17.35	11.87	17.41	17.76	16.37
b* (3)	6.11	8.07	5.17	5.29	9.09	5.38	4.30	5.08
L* AVERAGE	59.87	59.81	57.46	57.56	61.38	57.82	56.35	61.10
a* AVERAGE	15.34	14.67	16.62	17.47	12.37	17.00	18.31	14.89
b* AVERAGE	6.60	8.10	5.68	5.20	8.63	5.39	4.75	5.11



Date Collected	4/18/14	Day	4					
MOCON	Control 7C	Control 8C	Winpak 7	Winpak 8	Cryovac 7	Cryovac 8	Scavenger 7	Scavenger 8
Observations		Leaker						
MOCON - CO2 (%)	17.3	4.5	17.4	19.1	17.4	17.8	16.6	15.3
MOCON - O2 (%)	0.303	16.600	0.000	0.250	0.019	0.069	0.000	0.004
Colorimeter	Control 7C	Control 8C	Winpak 7	Winpak 8	Cryovac 7	Cryovac 8	Scavenger 7	Scavenger 8
L* (1) TOP	52.28	59.89	60.77	58.09	61.50	59.21	57.64	55.32
a* (1)	14.18	7.58	16.30	17.52	12.39	16.71	17.74	18.51
b* (1)	7.39	8.64	5.62	6.03	6.55	5.63	4.72	5.22
L* (2) MID	54.29	61.10	61.31	56.84	61.26	59.14	57.70	55.93
a* (2)	14.03	7.15	16.23	18.40	12.89	16.76	17.51	18.13
b* (2)	7.04	8.87	5.09	5.78	6.89	5.49	4.48	5.02
L* (3) END	55.80	61.41	61.64	57.01	60.32	58.84	58.40	54.62
a* (3)	14.74	7.09	15.64	18.17	13.84	17.12	17.46	18.71
b* (3)	7.14	9.29	5.15	5.82	7.90	6.13	5.10	5.08
L* AVERAGE	54.12	60.80	61.24	57.31	61.03	59.06	57.91	55.29
a* AVERAGE	14.32	7.27	16.06	18.03	13.04	16.86	17.57	18.45
b* AVERAGE	7.19	8.93	5.29	5.88	7.11	5.75	4.77	5.11
Date Collected	4/21/14	Day	7					
MOCON	Control 9C	Control 10C	Winpak 9	Winpak 10	Cryovac 9	Cryovac 10	Scavenger 9	Scavenger 10
Observations								
MOCON - CO2 (%)	17.4	17.9	10.8	18.1	17.1	16.4	15.2	15.4
MOCON - O2 (%)	0.187	0.086	0.000	0.000	0.033	0.002	0.000	0.000
Colorimeter	Control 9C	Control 10C	Winpak 9	Winpak 10	Cryovac 9	Cryovac 10	Scavenger 9	Scavenger 10
L* (1) TOP	57.40	59.06	56.10	59.71	59.59	57.17	53.70	57.52
a* (1)	12.02	14.09	16.50	16.11	9.52	15.81	19.55	17.10
b* (1)	7.31	6.67	5.37	6.17	6.66	6.74	5.70	5.63
L* (2) MID	59.40	60.46	55.30	58.45	58.66	56.78	55.88	57.77
a* (2)	12.12	12.74	17.53	16.85	9.81	16.33	17.92	16.46
b* (2)	7.21	7.23	5.93	5.89	6.57	5.39	5.65	4.32
L* (3) END	59.18	60.10	55.24	59.21	59.60	56.85	54.18	58.15
a* (3)	12.24	12.61	17.68	16.64	10.13	16.55	18.10	16.42
b* (3)	7.13	7.59	6.14	6.09	6.48	4.30	5.61	4.59
L* AVERAGE	58.66	59.87	55.55	59.12	59.28	56.93	54.59	57.81
a* AVERAGE	12.13	13.15	17.24	16.53	9.82	16.23	18.52	16.66
b* AVERAGE	7.22	7.16	5.81	6.05	6.57	5.48	5.65	4.85

## I.7 Wipak ferrous based oxygen scavenging film



**WINPAK**

### ESEV1E 1275 LOS3

#### DESCRIPTION:

This film was designed as a high barrier non-forming web with oxygen scavenger that helps extend shelf life in modified atmosphere applications.

#### COMPOSITION:

12 microns polyester/75 microns EVOH/linear low density polyethylene coextrusion with oxygen scavenger



- Polyester
- Adhesive
- Polyethylene
- Tie
- EVOH barrier
- Tie
- LLDPE sealant

Product Properties *		Units	Typical Values
Nominal Thickness		microns mils	87 3.5
Yield		g/m <sup>2</sup> in <sup>2</sup> /lb.	93.39 7528
Elongation	MD	%	90
	TD	%	60
Puncture Pointed Probe		lbs. N	4.0 18
Oxygen Transmission 24 hrs/23° C Dry		cc/sq. m cc/100 sq. in.	0.5 0.03
Moisture Vapor Transmission 24 hrs/37.8° C @ 90% R.H.		g/sq. m g/100 sq. in.	5.5 0.36

#### \* DISCLAIMER:

This data should be considered as average typical properties and not as a specification. This data is offered for information purposes and does not represent any type of guarantee or warranty of performance. Wipak assumes no liability for any incidents that may arise from the use of this data.

Letters of compliance to food packaging regulations are available upon request

v (2) 2010

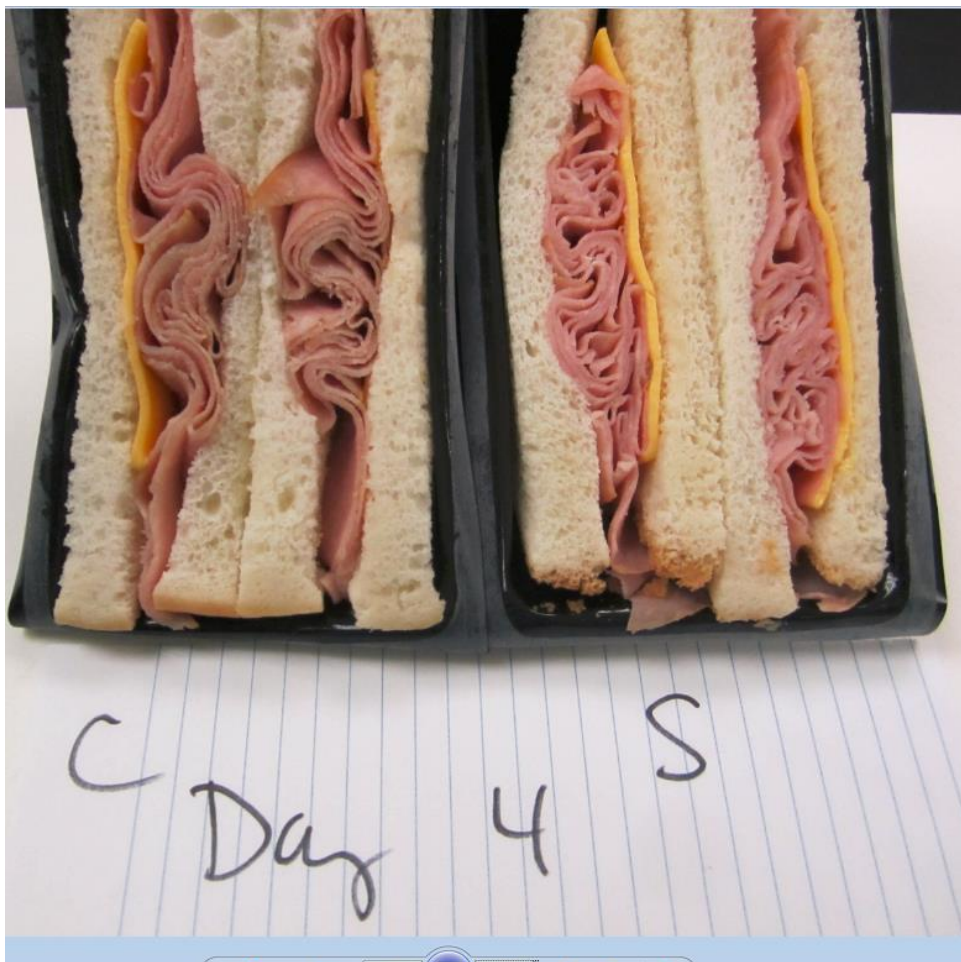
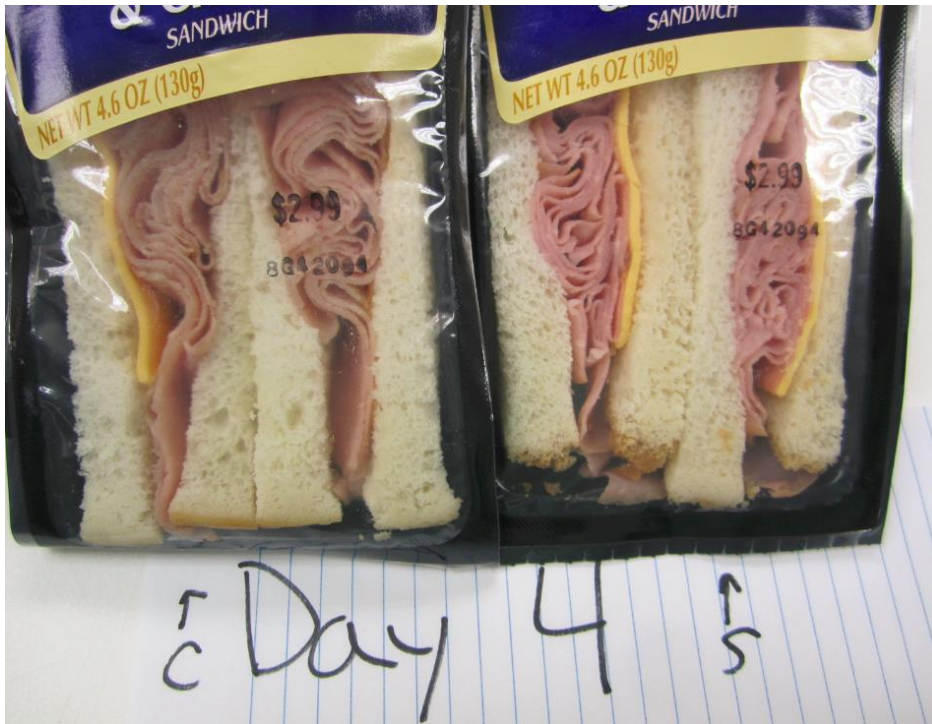
100 Sauftaux Cres., Winnipeg, MB, Canada R3J 3T3, Tel (204) 889-1015, Fax (204) 832-7781  
U.S. Mailing Address: P.O. Box 14748, Minneapolis, MN 55414



## Appendix J Test 10 Ferrous based oxygen scavenger packaging solutions and consumer study

J.1 Wedge format package day 4 as paired for consumer test (control left, scavenger sachet right)

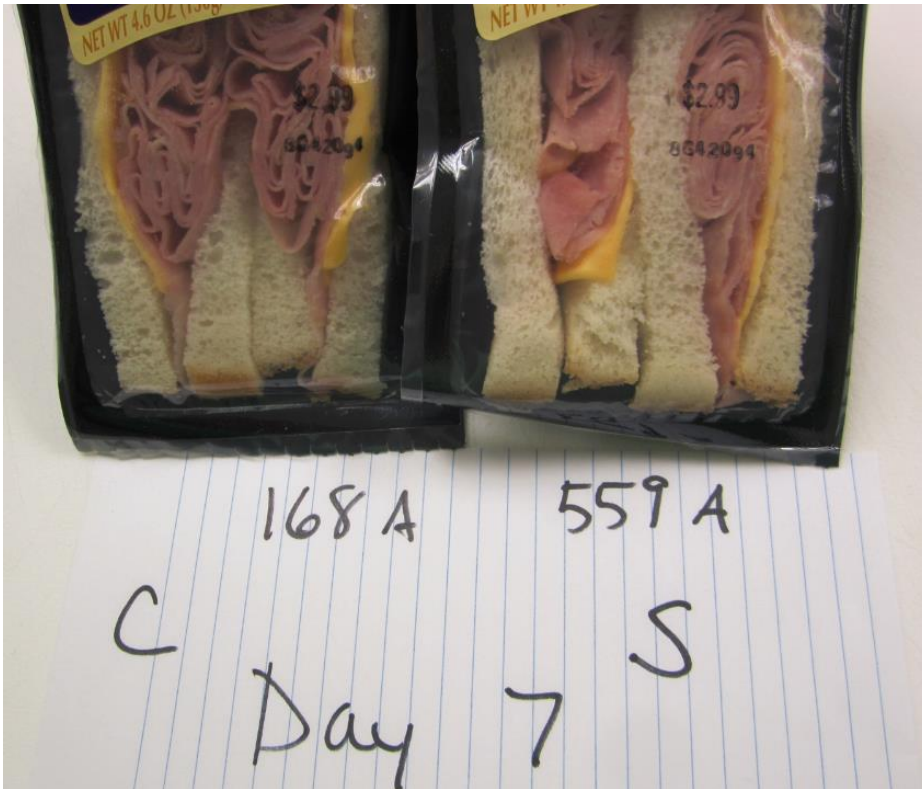






J.2 Wedge format package day 7 as paired for consumer test (control left, scavenger sachet right)

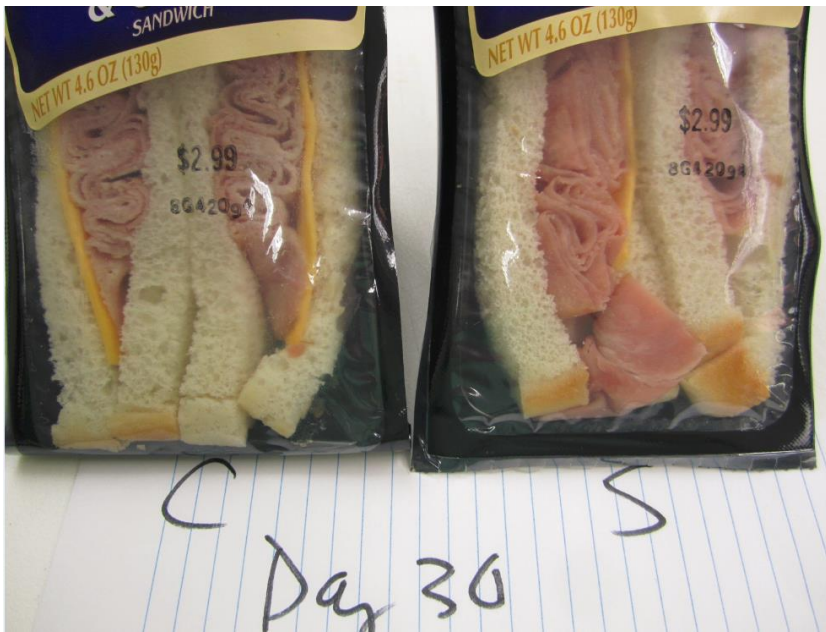




J.3 Wedge format package day 30 as paired for consumer test (control left, scavenger sachet right)









J.4 Flat meat format package day 1 as used for  $L^*a^*b^*$  analysis (control designated “C”, scavenger designated “S”)



J.5 Flat meat format package day 4 as used for  $L^*a^*b^*$  analysis (control designated “C”, scavenger designated “S”)



J.6 Flat meat format package day 7 as used for  $L^*a^*b^*$  analysis (control designated “C”, scavenger designated “S”)

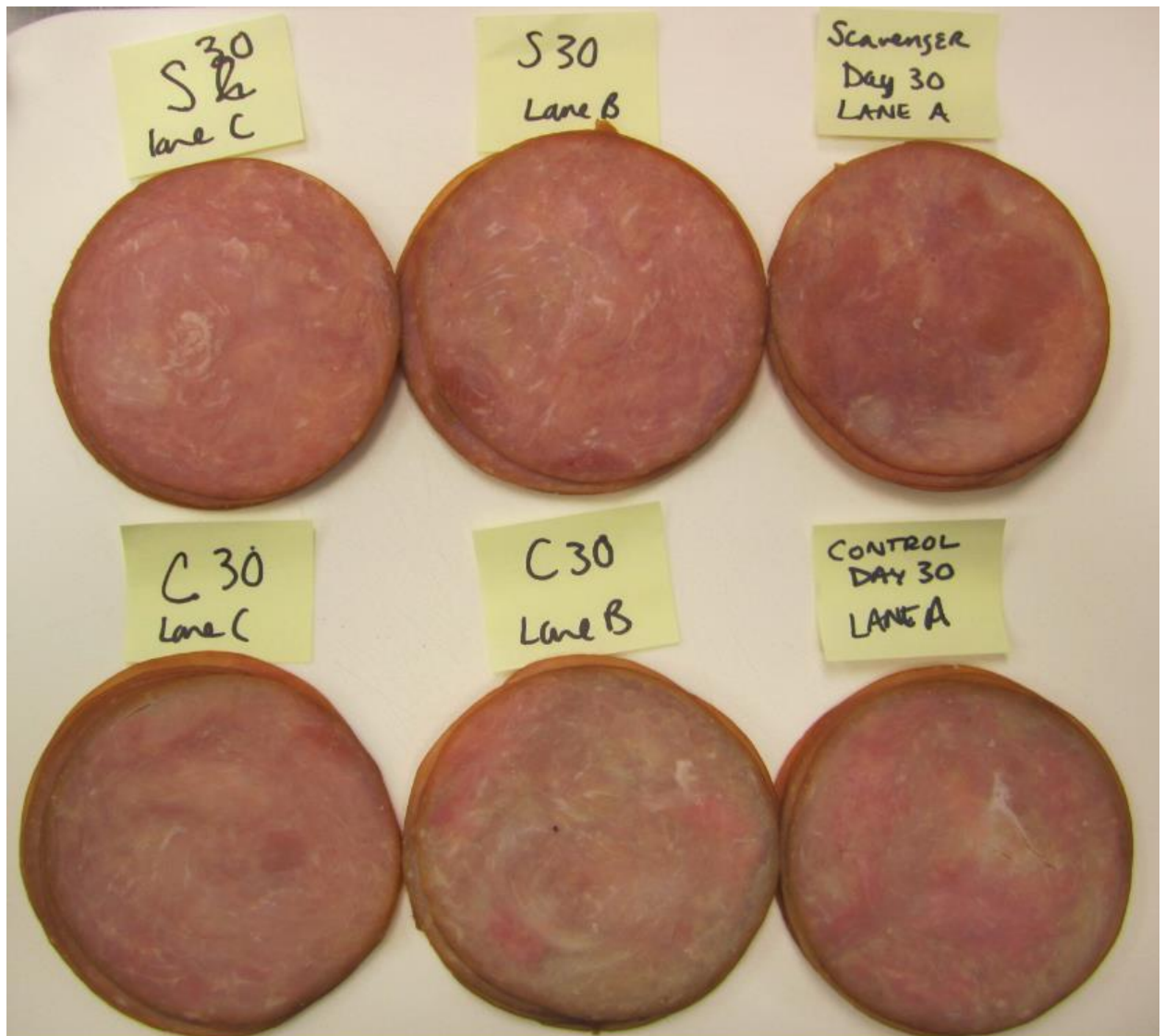


J.7 Flat meat format package day 14 as used for  $L^*a^*b^*$  analysis (control designated “C”, scavenger designated “S”)





J.8 Flat meat format package day 30 as used for  $L^*a^*b^*$  analysis (control designated “C”, scavenger designated “S”)



J.9  $L^*a^*b^*$  raw data Test 10

<b>Date Collected</b>	10/27/14 Day		30			
<i>MOCON</i>	C30 A	C30 B	C30 C	S30 A	S30 B	S30 C
<i>Observations</i>						
MOCON - CO2 (%)	15.6	15.6	15.6	12.6	13	13.4
MOCON - O2 (%)	0	0	0	0	0	0
<i>Colorimeter</i>	C30 A	C30 B	C30 C	S30 A	S30 B	S30 C
L* (1) TOP	58.64	59.31	59.09	54.38	60.02	59.06
a* (1)	13.33	11.00	14.65	17.72	16.03	18.32
b* (1)	7.78	6.81	6.57	6.51	5.83	5.93
L* (2) MID	58.64	59.75	58.57	52.89	59.16	61.23
a* (2)	14.28	10.77	13.96	18.35	17.10	17.46
b* (2)	6.32	6.56	6.01	6.36	5.96	5.98
L* (3) END	57.42	59.43	58.72	54.30	58.75	61.09
a* (3)	15.17	11.31	13.78	17.34	17.94	17.36
b* (3)	5.61	6.28	5.81	6.38	6.10	6.08
L* AVERAGE	58.23	59.50	58.79	53.86	59.31	60.46
a* AVERAGE	14.26	11.03	14.13	17.80	17.02	17.71
b* AVERAGE	6.57	6.55	6.13	6.42	5.96	6.00
OVERALL AVERAGE L*			58.84			57.88
OVERALL AVERAGE a*			13.14			17.51
OVERALL AVERAGE b*			6.42			6.13
<b>Date Collected</b>	10/27/14 Day		14			
<i>MOCON</i>	C14 A	C14 B	C14 C	S14 A	S14 B	S14 C
<i>Observations</i>						
MOCON - CO2 (%)	17.1	16.5	16.3	14.3	14.1	13.9
MOCON - O2 (%)	0.00	0.00	0.00	0.00	0.00	0.00
<i>Colorimeter</i>	C14 A	C14 B	C14 C	S14 A	S14 B	S14 C
L* (1) TOP	58.74	58.39	60.48	58.08	55.93	59.36
a* (1)	9.73	11.25	14.96	17.65	18.92	16.34
b* (1)	7.38	6.90	7.20	6.29	5.80	5.78
L* (2) MID	62.22	58.98	62.80	59.19	57.29	59.57
a* (2)	8.70	11.64	13.95	16.64	17.84	16.23
b* (2)	7.89	6.28	7.11	5.84	5.20	4.96
L* (3) END	60.43	59.36	63.21	59.24	58.88	59.90
a* (3)	9.52	11.27	13.68	16.73	17.20	16.36
b* (3)	7.63	6.26	7.23	5.52	5.77	4.70
L* AVERAGE	60.46	58.91	62.16	58.84	57.37	59.61
a* AVERAGE	9.32	11.39	14.20	17.01	17.99	16.31
b* AVERAGE	7.63	6.48	7.18	5.88	5.59	5.15
OVERALL AVERAGE L*			60.51			58.60
OVERALL AVERAGE a*			11.63			17.10
OVERALL AVERAGE b*			7.10			5.54

<b>Date Collected</b>	10/27/14 Day		7			
<b>MOCON</b>	C7 A	C7 B	C7 C	S7 A	S7 B	S7 C
<i>Observations</i>						
MOCON - CO2 (%)	16.8	16.6	16.9	15.6	16.0	15.8
MOCON - O2 (%)	0.0025	0.0525	0.155	0	0	0
<b>Colorimeter</b>	C7 A	C7 B	C7 C	S7 A	S7 B	S7 C
L* (1) TOP	60.12	60.63	59.33	61.37	59.55	59.82
a* (1)	11.26	14.66	15.34	16.02	16.90	17.21
b* (1)	8.36	7.19	6.92	5.30	5.82	5.28
L* (2) MID	61.57	60.43	61.13	61.61	58.95	61.31
a* (2)	10.46	15.18	14.68	15.90	17.59	16.41
b* (2)	8.05	7.06	6.29	5.37	6.05	5.13
L* (3) END	60.23	58.30	60.55	60.06	58.20	60.97
a* (3)	11.42	15.84	14.60	16.83	17.90	16.28
b* (3)	8.03	7.53	6.88	6.00	6.00	5.30
L* AVERAGE	60.64	59.79	60.34	61.01	58.90	60.70
a* AVERAGE	11.05	15.23	14.87	16.25	17.46	16.63
b* AVERAGE	8.15	7.26	6.70	5.56	5.96	5.24
OVERALL AVERAGE L*			60.25			60.20
OVERALL AVERAGE a*			13.72			16.78
OVERALL AVERAGE b*			7.37			5.58
<b>Date Collected</b>	10/27/14 Day		4			
<b>MOCON</b>	C4 A	C4 B	C4 C	S4 A	S4 B	S4 C
<i>Observations</i>						
MOCON - CO2 (%)	18.7	16.9	17	16	16.3	16.3
MOCON - O2 (%)	0	0.0011	0.0184	0	0	0
<b>Colorimeter</b>	C4 A	C4 B	C4 C	S4 A	S4 B	S4 C
L* (1) TOP	59.20	60.21	63.30	56.92	60.96	62.16
a* (1)	9.82	13.63	13.43	18.67	17.10	15.10
b* (1)	9.87	7.24	7.70	5.72	5.77	6.91
L* (2) MID	59.40	59.82	65.69	58.33	59.69	62.60
a* (2)	10.97	14.16	12.35	17.87	17.45	15.54
b* (2)	8.97	7.30	8.19	5.70	5.36	6.66
L* (3) END	58.63	60.45	63.55	58.14	59.17	61.01
a* (3)	12.14	14.18	13.49	18.24	17.44	16.32
b* (3)	8.47	7.38	7.84	5.63	5.31	6.53
L* AVERAGE	59.08	60.16	64.18	57.80	59.94	61.92
a* AVERAGE	10.98	13.99	13.09	18.26	17.33	15.65
b* AVERAGE	9.10	7.31	7.91	5.68	5.48	6.70
OVERALL AVERAGE L*		59.62		60.99		60.93
OVERALL AVERAGE a*		12.48		15.68		16.49
OVERALL AVERAGE b*		8.21		6.80		6.09
<b>Date Collected</b>	10/27/14 Day		1			
<b>MOCON</b>	C1 A	C1 B	C1 C	S1 A	S1 B	S1 C
<i>Observations</i>						
MOCON - CO2 (%)	17.4	17.0	17.1	17.1	16.5	16.7
MOCON - O2 (%)	0.0082	0.0506	0.0777	0	0	0
<b>Colorimeter</b>	C4 A	C4 B	C4 C	S4 A	S4 B	S4 C
L* (1) TOP	56.52	55.72	57.58	59.36	57.47	61.34
a* (1)	15.00	19.57	17.03	18.05	18.43	16.86
b* (1)	7.38	7.33	5.70	6.31	6.31	6.70
L* (2) MID	59.15	57.23	57.80	60.78	57.20	61.11
a* (2)	14.02	18.72	16.89	17.39	19.03	17.51
b* (2)	7.71	7.04	5.46	5.99	6.16	6.72
L* (3) END	60.09	56.61	57.13	62.71	56.87	59.44
a* (3)	13.02	18.18	17.39	16.13	19.01	18.43
b* (3)	8.11	7.35	5.59	6.32	6.05	6.83
L* AVERAGE	58.59	56.52	57.50	60.95	57.18	60.63
a* AVERAGE	14.01	18.82	17.10	17.19	18.82	17.60
b* AVERAGE	7.73	7.24	5.58	6.21	6.17	6.75
OVERALL AVERAGE L*		57.55		59.23		58.91
OVERALL AVERAGE a*		16.42		17.15		18.21
OVERALL AVERAGE b*		7.49		5.90		6.46

J.10 Quality control checks for oxygen and carbon dioxide for the production line  
designated Red Phoenix on 7/28/14

Revised: 7/3/13  
Supersedes: 2/4/11  
SW73A

**EAS**  
E.A. Sween Company

Deli Express/E.A. Sween Co.  
16101 West 78th Street  
Eden Prairie, MN 55344  
1-800-328-8184 FAX 952-937-0186

**MAP CHECK SHEET - ROUND PACKAGING**

Line RT Mocon ID M-C

Multivac sl meat & patty sandwiches: Oxygen (O2) target .2% or less; Carbon Dioxide (CO2) 17 - 28%  
Multivac Salad sandwiches: Oxygen (O2) target .20% or less; Carbon Dioxide (CO2) target 0 - .1%

Oxygen (O2) calibration checked and found to be acceptable ☒  
Carbon dioxide (CO2) calibration checked and found to be acceptable ☒

Product: SLBBL Time: 7:10

CO2	O2	CO2	O2	CO2	O2
20.0	PM	19.7	PM	19.8	20
20.0	PM	19.9	20	19.9	20

Comments:

Product: SLBBL Time: 8:05

CO2	O2	CO2	O2	CO2	O2
21.0	13	20.3	18	19.6	19
19.8	20	20.2	14	20.2	20

Comments:

Product: SLBBL Time: 7:14am

CO2	O2	CO2	O2	CO2	O2
20.2	20	19.7	PM	19.7	PM
19.8	PM	19.4	PM	19.6	PM

Comments:

Product: SLBBL Time: 11:50

CO2	O2	CO2	O2	CO2	O2
19.7	19	19.7	14	19.2	11
19.8	PM	19.0	15	19.0	10

Comments:

Product: SLBBL Time: 7:35

CO2	O2	CO2	O2	CO2	O2

Comments:

Product: SLBBL Time: 9:55


CO2	O2	CO2	O2	CO2	O2
19.3	12	19.2	21	19.1	11
19.7	1	19.5	12	19.7	22

Comments:



J.11 Quality control checks for oxygen and carbon dioxide for the production line  
designated Green Wolf on 7/28/14 (a "squiggly line" was used to indicate ppm values for the O<sub>2</sub>%)

Revised: 7/3/13  
Supersedes: 2/4/11  
SW73B

  
**E.A. Sween Company**  
**MAP CHECK SHEET**

Dell Express/E.A. Sween Co.  
16101 West 78th Street  
Eden Prairie, MN 55344  
1-800-328-8184 FAX 952-937-0186

Line 7-28-14 Mocon ID 6-A

Multivac meat & patty sandwiches: Oxygen (O<sub>2</sub>) target .2% or less; Carbon Dioxide (CO<sub>2</sub>) 17 - 28%  
 Multivac Salad sandwiches: Oxygen (O<sub>2</sub>) target .20% or less; Carbon Dioxide (CO<sub>2</sub>) target 0 - .1%  
 Oxygen (O<sub>2</sub>) calibration checked and found to be acceptable \_\_\_\_\_  
 Carbon dioxide (CO<sub>2</sub>) calibration checked and found to be acceptable \_\_\_\_\_

Product: Bologna Time: 1:34

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
213	~	211	~	211	~	210	~
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
216	~	210	~	214	~	209	~

Comments:

Product: Bologna Time: 1:34

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
217	~	207	~	212	~	216	~
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
209	~	205	~	203	~	205	~

Comments:

Product: Sausage Time: 2:07

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
217	~	214	~	217	~	210	~
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
209	~	210	~	209	~	205	~

Comments:

Product: Sausage Time: 1:40

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
200	~	213	~	215	~	207	~
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
203	~	216	~	212	~	203	~

Comments:

Product: Sausage Time: 2:25

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
✓	✓	✓	✓	✓	✓	✓	✓
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
✓	✓	✓	✓	✓	✓	✓	✓

Comments:

Product: Sausage Time: 2:10

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
✓	✓	✓	✓	✓	✓	✓	✓
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
✓	✓	✓	✓	✓	✓	✓	✓

Comments:

Product: Sausage Time: 2:10

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
210	~	214	~	215	~	219	~
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
217	~	210	~	214	~	216	~

Comments:

Product: Sausage Time: \_\_\_\_\_

CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
213	~	208	~	211	~	216	~
CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
210	~	213	~	204	~	210	~

Comments:

Project 845 - Thermostat

## J.12 CLT consumer screening questionnaire

09935-DE – Meat Discoloration Visual CLT – Screener

Visual Packaging Test in Plymouth on Monday, October 27, 2014.

*For studies recruited from FPI's respondent database, all respondents are prescreened for age, gender, and past participation. Only those within the project specified age range, gender and past participation requirements will be provided individual access to complete the survey.*

### Pre-Screening Criteria:

Age:	18 to 54 years old
Gender:	50:50 Male:Female
Past Participation:	No participation in past 60 days

**Bold/Highlight=Continue. Other programming notes in [brackets]**

1 This survey is for \${firstname}. Are you \${firstname}?  
(Select one)

**Yes**  
No

**[Must select a bold option to continue]**

2 Sometimes we are looking for people who work in certain industries. Are you, any member of your household, relatives, or any close friends employed by...?  
(Select all that apply)

**A car manufacturer**  
**A car rental company**  
A food manufacturer or processor  
A food wholesaler distributor  
**A government agency**  
A market research company  
A newspaper, TV, or radio station  
**A telecommunications company**  
An advertising agency  
**None of the above**

**[Must select only bold options to continue]**

3 Do you have any food allergies, sensitivities, restrictions, or intolerances?  
(Select one)

Yes

**No**

**[Must select a bold option to continue]**

4 This test will involve looking at several visual samples. Do you have any color blindness or color vision deficiency?  
(Select one)

Yes

**No**

**[Must select a bold option to continue]**

5 Which of the following best describes your ethnic background?  
(Select one)

**American Indian/Alaska Native**

**Asian**

**Black/African American**

**Hispanic or Latino**

**Native Hawaiian/Other Pacific Islander**

**White/Caucasian**

**Other**

**[Must select a bold option to continue]**

6 In the past 3 months, which of the following prepared food items have you purchased and eaten from a restaurant, grocery store, convenience store, mini-mart, or vending machine? (Select all that apply)

Pizza  
Hot dog  
**Sandwich**  
Burrito  
Breakfast sandwich  
Salad  
Chicken tenders  
Nachos  
None of the above

[Randomize. Must select a bold option to continue]

7 You mentioned that you have purchased and eaten sandwiches in the past 3 months. How often do you purchase and consume the following types of sandwiches?  
(Select one answer per row)

	Once a week or more	Two to three times a month	Once a month	Once every 2 to 3 months	Once every 4 to 6 months	Less frequently than every 6 months
Sandwiches you prepare yourself at home	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sandwiches prepared at a restaurant	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Prepared sandwiches from a grocery store	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Fresh, pre-packaged sandwiches from a convenience store, such as breakfast sandwiches, hamburgers, or deli meat sandwiches</b>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Prepared sandwiches from a vending machine	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

[Randomize. Must select a highlighted option to continue]

8 You mentioned fresh, pre-packaged sandwiches from a convenience store. Which of the following stores have you purchased pre-packaged sandwiches from in the last month?  
(Select all that apply)

Holiday gas station

Super America

BP gas station

Kwik Trip

Freedom gas station

Other (Please specify): \_\_\_\_\_

[Must select a bold option to continue]

9 Which, if any, of the following flavors/varieties of deli sandwiches do you like and are interested in trying?

(Select all that apply)

**Ham**

Turkey

Chicken

Roast beef

**Cured meat, such as salami, pepperoni, or prosciutto**

None of the above

**[Must select both bold options to continue]**

10 Which, if any, of the following types of sandwiches would you be willing to try?

(Select all that apply)

Smoked Turkey on White Bread

**Ham and Cheese on White Bread**

Italian Meat on Focaccia Bread

Egg, Sausage, and Cheese on an English Muffin

Italian Meat Sub Sandwich

Sausage & Biscuit Sandwich

Chicken and Pesto on Ciabatta Bread

None of the above

**[Must select a bold option to continue]**

11 Would you describe yourself as being “shy” in groups?

(Select one)

**Yes**

Sometimes

**No**

**[Must select a bold option to continue. Must select a highlighted option to be eligible for peel off session.]**

12 How comfortable are you sharing your opinion in a group setting?  
(Select one)

Not very comfortable sharing my opinion

Most of the time not comfortable sharing my opinion

Some of the time comfortable sharing my opinion

**Most of the time comfortable sharing my opinion**

**Always very comfortable sharing my opinion**

[Must select a bold option to continue. Must select a highlighted option to be eligible for peel off session.]

## J.13 Moderator script for peel off session

09935-DE Meat Discoloration CLT  
Peel Off Discussion Guide Draft v1  
Date: 10/8/14

**Business Objective:** To protect Deli Express share of cured meat sandwiches by addressing customers concerns about discoloration.

**Research Objectives:**

1. To determine which sample is preferred between the current and prototype pairs and degree of preference.
2. To determine if new packaging solution will meaningfully increase purchase interest and liking of Ham Single-Wedge Sandwiches.
3. To understand consumer's likes/dislikes about antioxidant scavengers.

### A. INTRO

**INTRO:** Hello and welcome! My name is Kristen and I am the moderator for our 45 minute focus group. Thank you so much for agreeing to stay for some additional questions. Your opinions are important to us.

This is a free flowing discussion and there are no wrong answers. I'm looking for your opinion, even if someone else in the group does not agree with you.

I work independently and I'm working on this project as a consultant.

**DISCLOSURES:** There are microphones and a camera taping us today. This is mostly so I don't have to take extensive notes to write an accurate report— not of who said what but "what got said."

Behind the mirror, there may be an observer or two.

**PERMISSIONS:** At anytime you can excuse yourself to use the restroom. I ask that only one person should be up or out at a time.

**GUIDELINES:** In order to make this research session work the best, here are some guidelines, mostly so that my audio recorder and I capture what you said (will also be posted, so we can go through faster):

- Please talk one at a time
- Talk in a voice as loud as mine
- Avoid side conversations with your neighbors
- I'd like to hear from everyone; however, you don't need to answer every question
- Work for equal "air time" so that no one talks too little or too much
- Allow for different points of view. There are no wrong answers
- Say what you believe, whether or not anyone else agrees with you



- I may have to cut you off to make sure that we are able to cover everything we would like to cover today
- Please turn off your cell phones

INTRO & WARM UP: Please introduce yourself to the group and tell us:

- Name
- Favorite Prepared Sandwich

#### 1. Tasting

1. First, we are going to try two sandwiches. In front of you I have a piece of paper where I'd like you to mark your impressions of the sandwich. Let's start with code XYZ. Please fill out the first sheet of paper and follow the order of the questions there. First, you are asked to visually evaluate the product prior to tasting, however that would be normally for you, and then taste it. Then we ask you one additional question at the end.

Let's discuss what your impressions of this sandwich were.

- A. What are your thoughts on how it looked, before you ate it?
  - a. Listen for color of the meat
  - b. Probe, if brought up: What tells you the meat is fresh?
- B. How did it taste?
- C. What were your impressions of the packaging?
  - a. Listen and probe on scavenger packet for the sample that has the packet.

2. Now, we are going to try the second sandwich. Turn your sheet of paper over to the next sheet in your packet. While the questions are the same, we'd like you to pretend that this is the first sandwich you've tried, so try not to compare and contrast the sandwiches to each other in this part.

Again, we will discuss your impressions of this sandwich.

- A. What are your thoughts on how it looked, before you ate it?
  - a. Listen for color of the meat
- B. How did it taste?
- C. What were your impressions of the packaging?
  - a. Listen and probe on scavenger packet for the sample that has the packet.

#### 2. Spectrum

3. Next we have a group activity. I'm going to put you into two small groups, and I'd like to know which of these sandwiches looks the most appealing to eat. [Ends of the spectrum are: Least likely to purchase to most likely to purchase].

- A. Help me understand how you ranked these sandwiches? What is behind your thinking?
- B. For those that are least likely to purchase – how unlikely is it for you to purchase? What is the degree of your conviction? Is it, "I wouldn't buy this" or is it "It wouldn't be my first choice but I would buy it."

<b>3. Scavenger Packets</b>
-----------------------------

4. I'd like to ask you a few more questions about the packaging. As you saw with one of the sandwiches, there is a little packet on here. What are your thoughts on this packet being included in your sandwiches from now on?

- A. What are some of the reasons why you think they would include this in the packaging?
- B. What are any of the downsides for you, if any?

5. That is all my questions! Do you have anything else to add based on our discussion today?

**Tasting Ballots**

Please write the code of the sample you are trying

My thoughts on how this sample looks, before eating it

My thoughts on how this sample tastes

My thoughts on the packaging

**STOP, DO NOT TURN YOUR PAGE OVER UNTIL THE MODERATOR INSTRUCTS YOU TO.**

For this second sample, please try and forget what you thought about the first sample.  
Pretend you are evaluating it as if it was the first time you tried it.

Please write the code of the sample you are trying

My thoughts on how this sample looks, before eating it

My thoughts on how this sample tastes

My thoughts on the packaging

STOP, DO NOT TURN YOUR PAGE OVER UNTIL THE MODERATOR INSTRUCTS YOU TO.

Preference

Think back to both samples. Which sandwich did you prefer? Please write the code below.

What are your reasons for picking the sandwich you did?

J.14 Consumer questionnaire for preference test

R#

Respondent No.

**VISUAL PRODUCT EVALUATION**

Your Name: \_\_\_\_\_

- Please use the pen provided.
- Answer all questions in the order asked on the questionnaire.
- Place your mark in the box. ☐
- Mark only one box per question.
- Make no stray marks on the questionnaire.
- Cross out any answers you wish to change and write "no" next to the incorrect answer.

R#

S1, Code:

Please LOOK at the sample and answer the following questions.

xx. OVERALL, how well do you LIKE or DISLIKE the APPEARANCE of this product?

☐ Dislike ☐ Dislike ☐ Dislike ☐ Dislike ☐ Neither Like ☐ Like ☐ Like ☐ Like ☐ Like  
Extremely Very Much Moderately Slightly Nor Dislike Slightly Moderately Very Much Extremely

xx. How much do you LIKE or DISLIKE the MEAT COLOR of this product?

☐ Dislike ☐ Dislike ☐ Dislike ☐ Dislike ☐ Neither Like ☐ Like ☐ Like ☐ Like ☐ Like  
Extremely Very Much Moderately Slightly Nor Dislike Slightly Moderately Very Much Extremely

xx. Rate the MEAT COLOR of this product.

☐ Much Too ☐ Somewhat ☐ Just About ☐ Somewhat ☐ Much Too  
Light Too Light Right Too Dark Dark

xx. If this product were available where you shop, how likely would you be to buy it for your household.

☐ Definitely ☐ Probably ☐ Might or Might ☐ Probably ☐ Definitely  
Would Not Buy Would Not Buy Not Buy Would Buy Would Buy

xx. Overall, how well does this product meet your EXPECTATIONS of a pre-packaged sandwich?

☐ Much Worse ☐ Somewhat Worse ☐ About As ☐ Somewhat Better ☐ Much Better  
Than Expected Than Expected Expected Than Expected Than Expected

R#



**PLEASE WAIT UNTIL THE MODERATOR TELLS YOU TO  
TURN TO THE NEXT PAGE.**



R#

xx. Which **ONE** of the two samples do you prefer OVERALL?  
(Check **ONE** product code number below.)

☐ Prefer Product Seen 1st (Code **XXX**)

☐ Prefer Product Seen 2nd (Code **XXX**)

xx. What is the main reason you preferred this sample?


R#

# DEMOGRAPHICS

This information is confidential and will be used for classification purposes only.

xx. What is your gender?

☐

Male

☐

Female

xx. Which category includes your age?

☐

17 or younger

☐

18–24

☐

25–35

☐

36–46

☐

47–55

☐

56–64

☐

65 or older

xx. What is your ethnic background?

☐

American  
Indian/Alaska  
Native

☐

Asian

☐

Black/  
African  
American

☐

Hispanic or  
Latino

☐

Native  
Hawaiian/Other  
Pacific Islander

☐

White/  
Caucasian

☐

Other:  
(Specify  
\_\_\_\_\_)

xx. What is the last level or grade of school that you completed?

☐

Some grade  
school or high  
school

☐

High school  
graduate

☐

Some college  
or technical  
school

☐

2-year college  
/ technical  
degree

☐

4-year college  
degree

☐

Some graduate  
work

☐

Graduate  
degree

xx. Including yourself, how many people are living in your household? This includes babies but does not include students living away from home.

☐

1

☐

2–3

☐

4–5

☐

6–7

☐

8 or more

xx. Which group best represents your total yearly household income (before taxes)?

☐

Under  
\$25,000  
per year

☐

\$25,000–  
\$39,999  
per year

☐

\$40,000–  
\$59,999  
per year

☐

\$60,000–  
\$79,999  
per year

☐

\$80,000–  
\$99,999  
per year

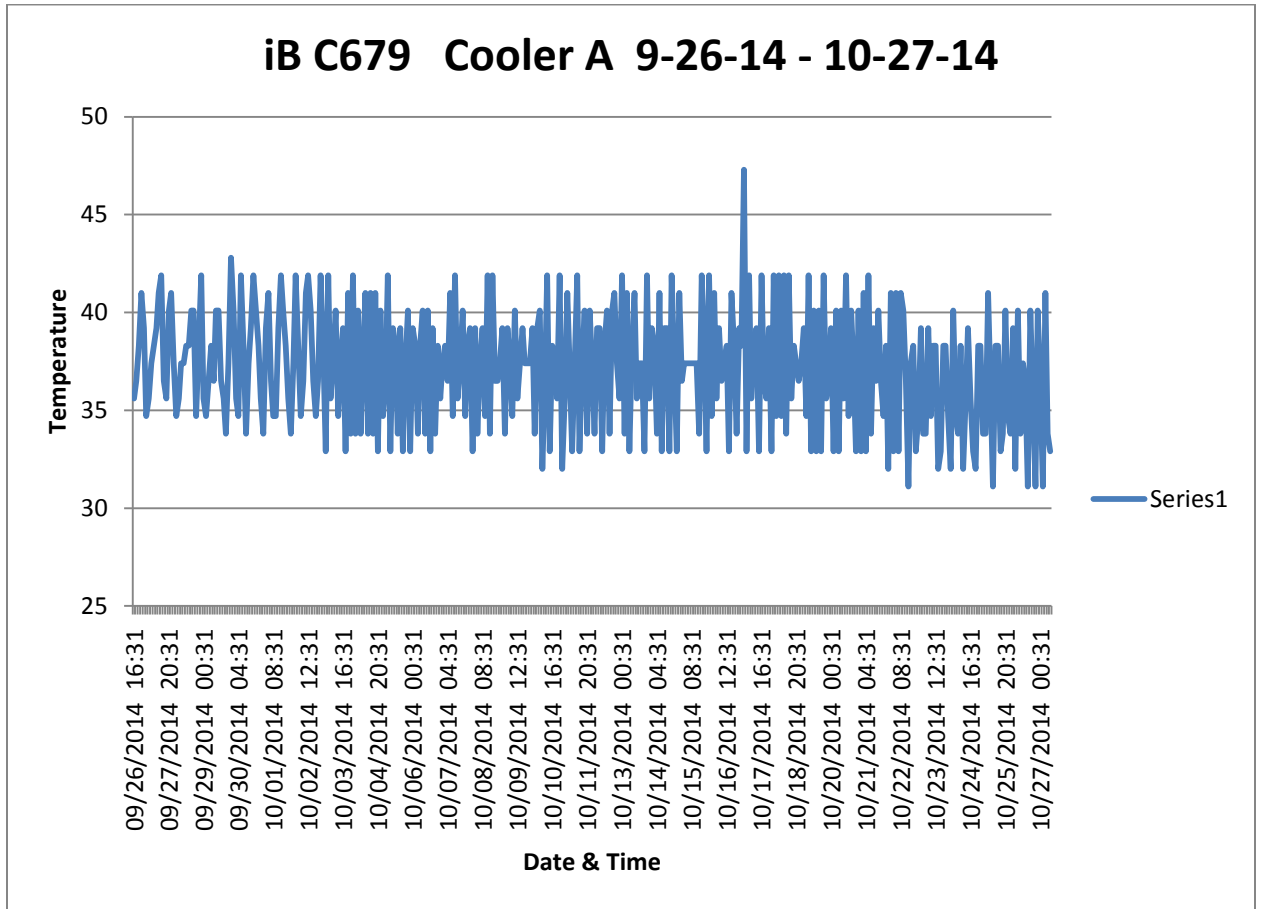
☐

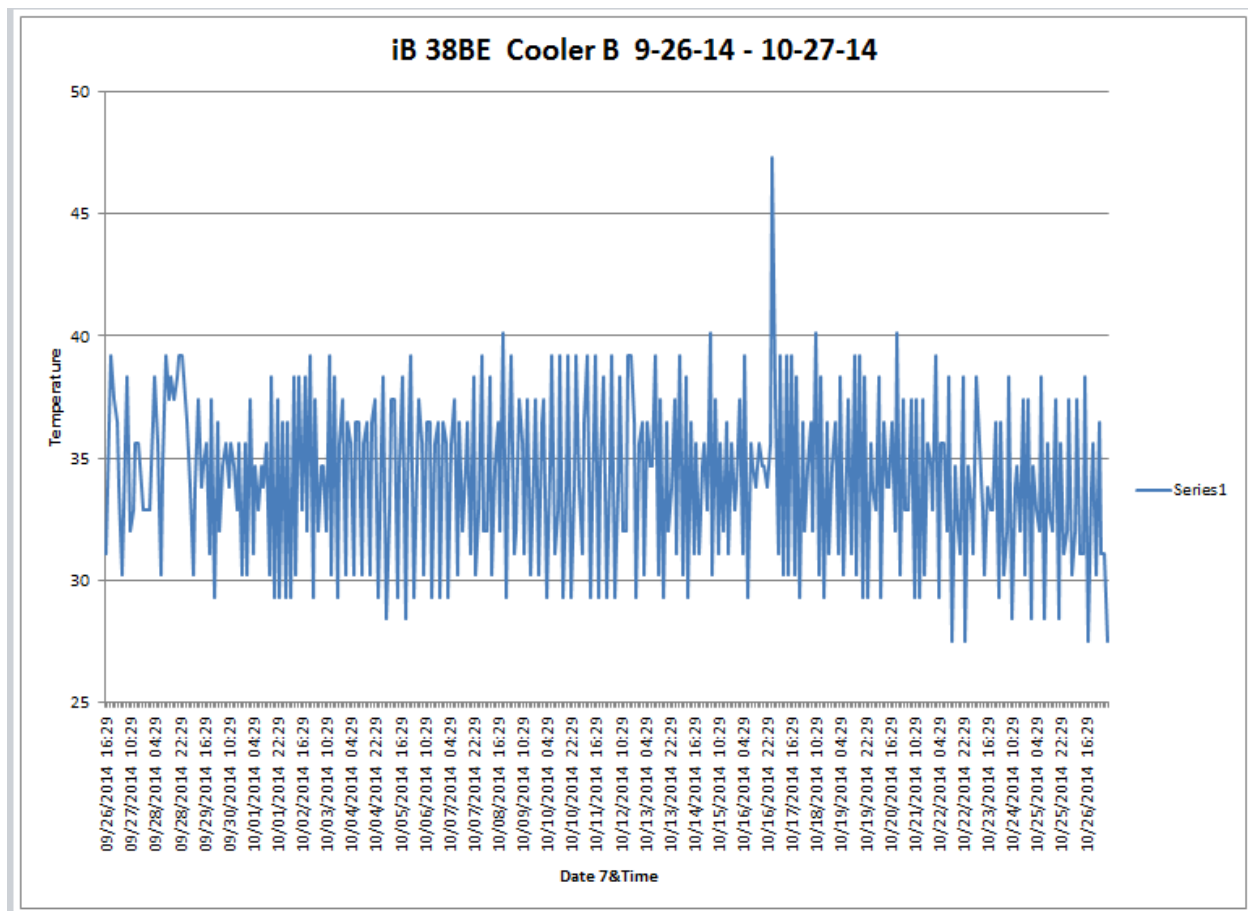
\$100,000  
or more  
per year

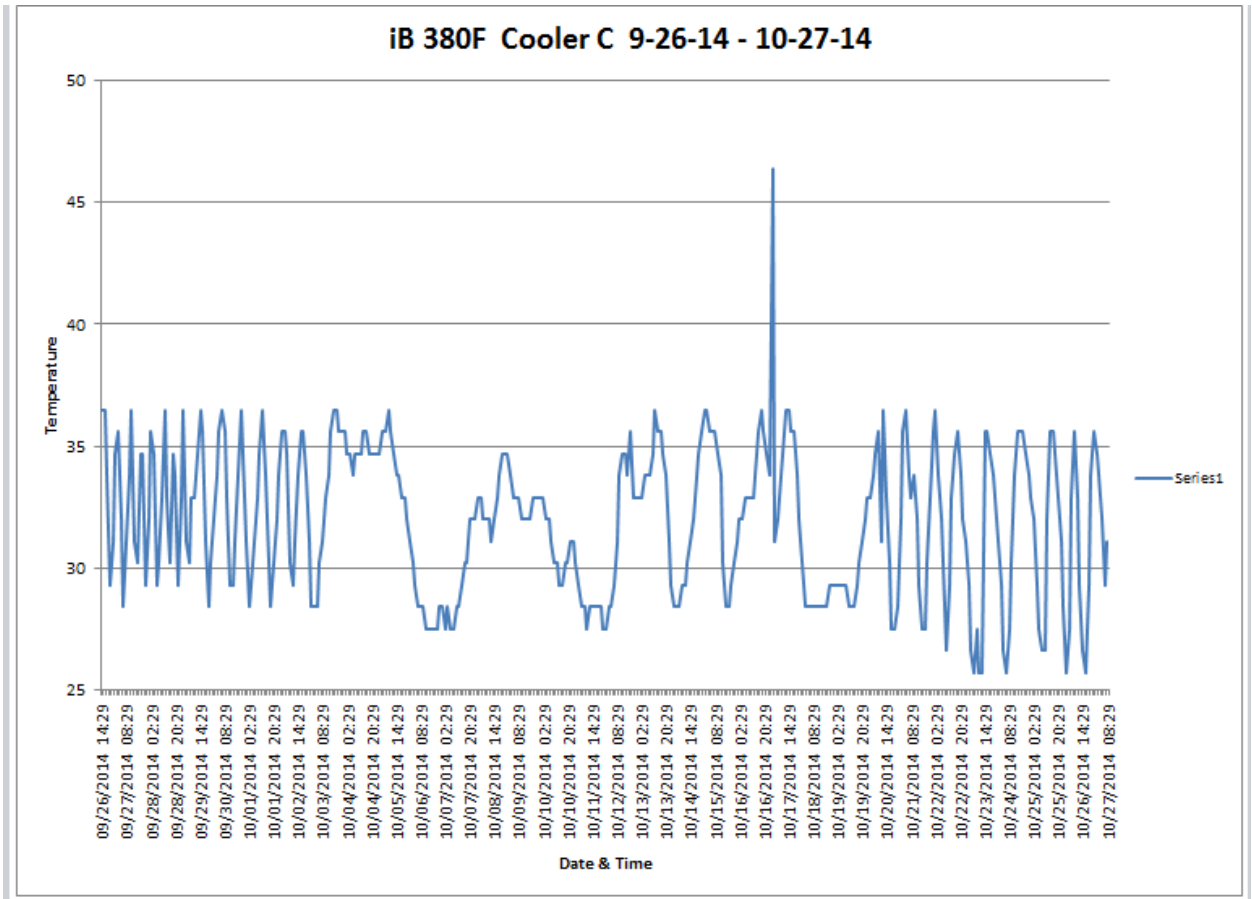
☐

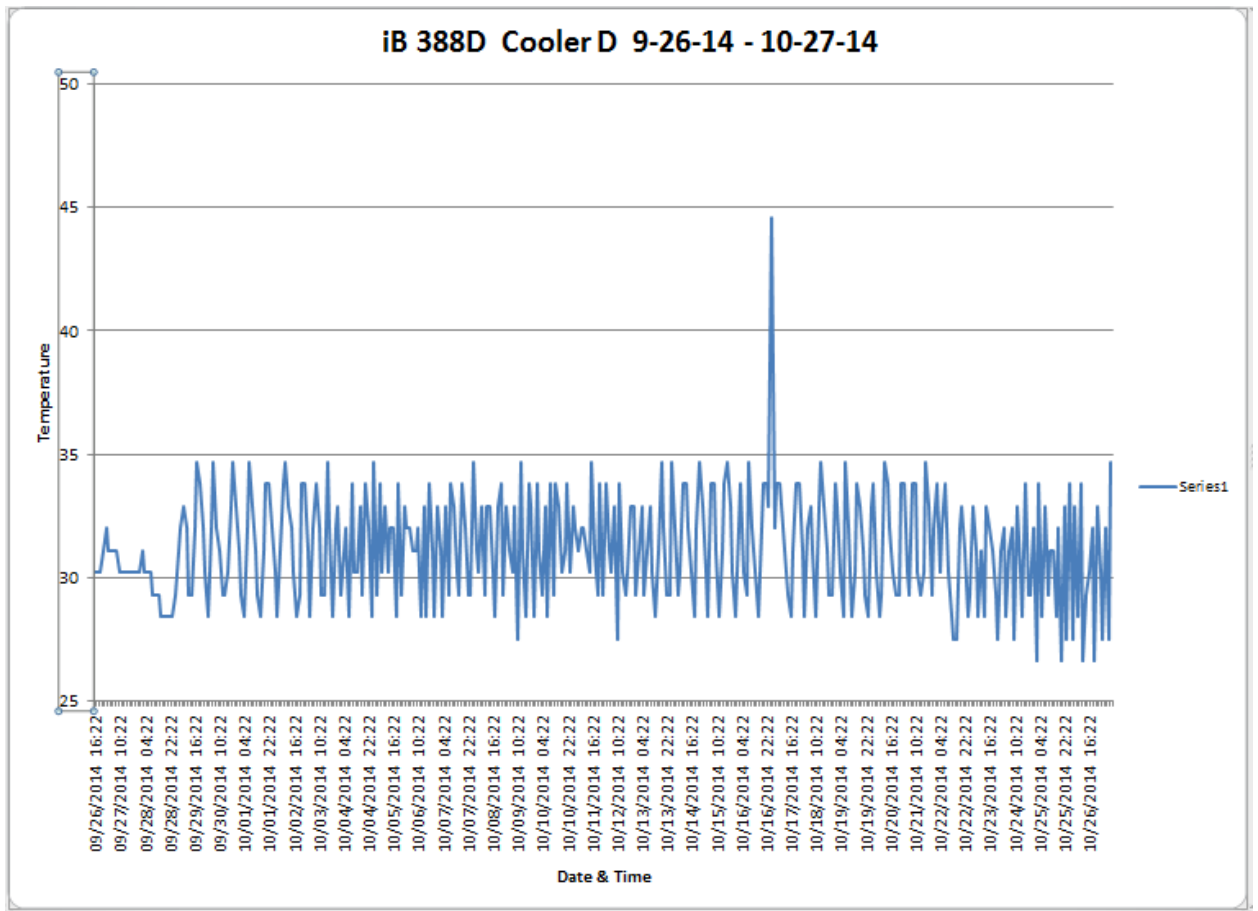
Prefer not  
to answer

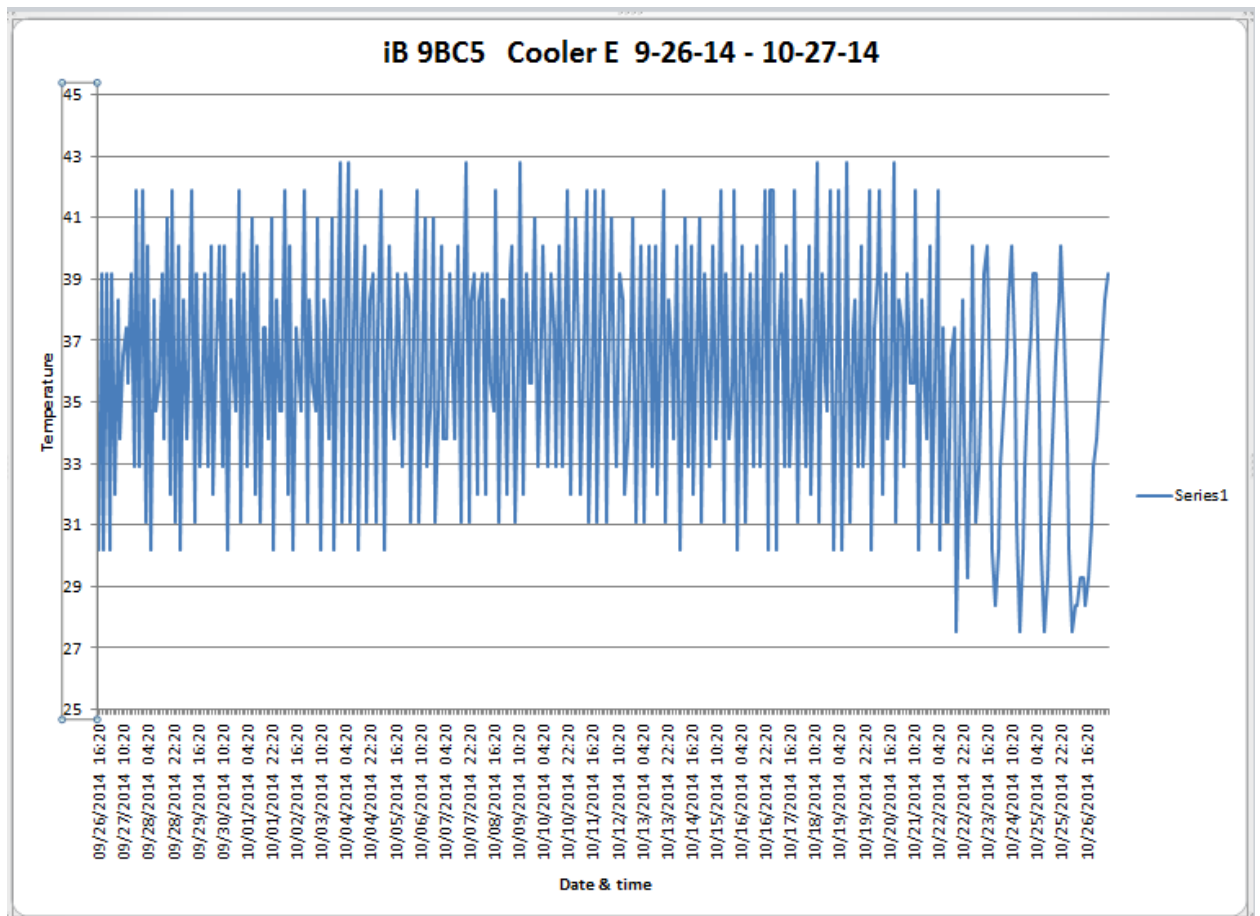
J.15 Temperature charts from coolers in test 10 (reported in Fahrenheit)

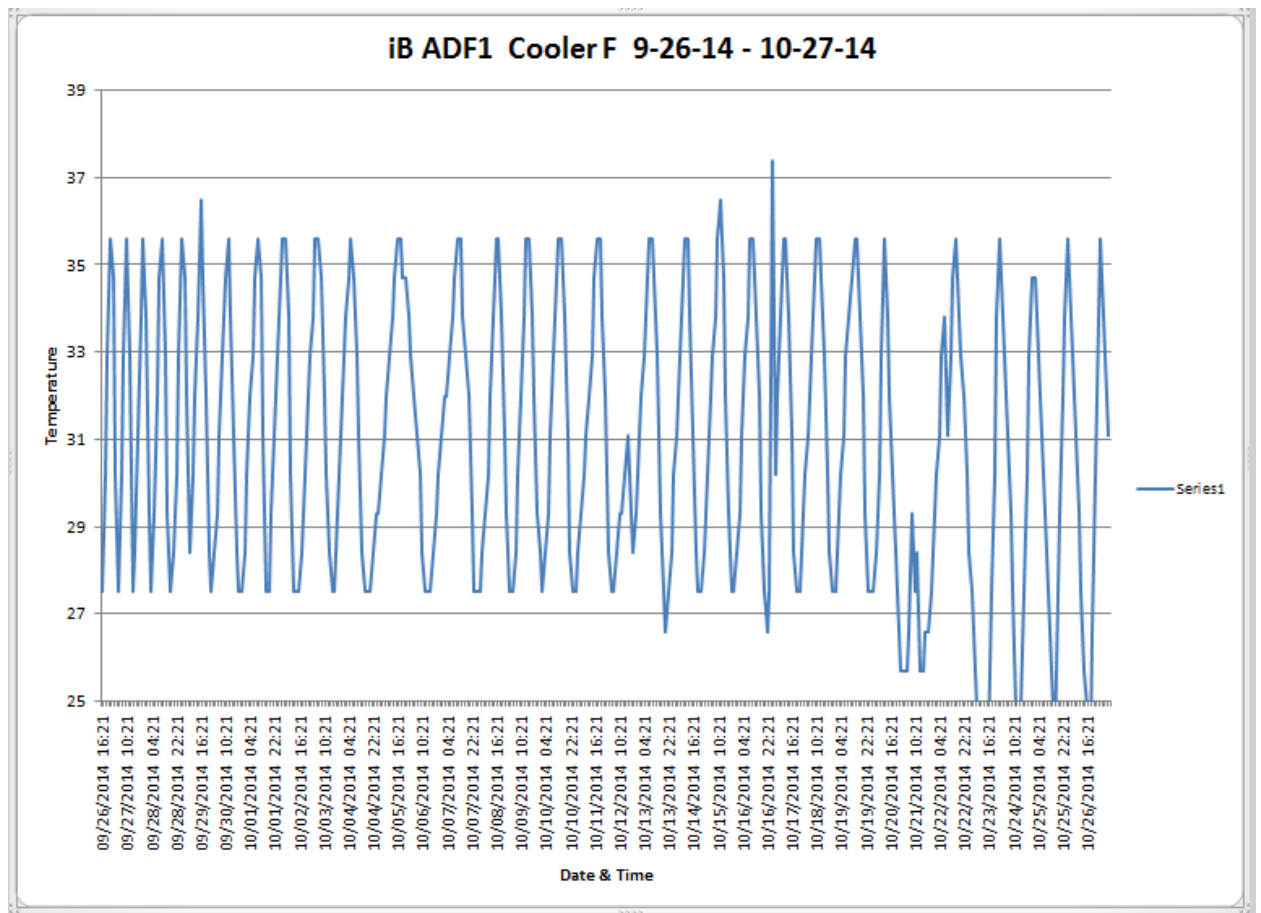














## J.16 Test design summary

DocuSign Envelope ID: 42AC80AD-EDF2-4471-8183-EB4BA106766F

### E.A. Sween Company Meat Discoloration Visual CLT (3 pairs) + Peel-offs **Revision 3**

Bid Date: September 24, 2014  
Client Contact: Grant Nellis  
E. A. Sween Company  
16101 West 78<sup>th</sup> Street  
Eden Prairie, MN 55344  
gnellis@easween.com

#### STUDY AT A GLANCE

Research Objective:	1. To determine which sample is preferred between the current and prototype pairs <b>and degree of preference.</b> 2. To determine if new packaging solution will meaningfully increase purchase interest and liking of Ham Single-Wedge Sandwiches. 3. To understand consumer's likes/dislikes about antioxidant scavengers.
Action Standard:	Purchase Interest, Preference, and Visual Liking $\geq$ current
Study Type:	Visual Central Location Test (CLT) + Peel-offs (Blind)
Products Tested:	Ham Single-Wedged Sandwiches (Current vs Prototype at Day <b>4, 7, 30</b> )
Products per Respondent:	<b>All respondents will see all 3 pairs, sequential monadic, fully rotated and balanced</b>
Peel-off Group Orientation:	Four groups of 5 respondents- mixture of respondents that chose current and prototype with scavenger; <b>The peel-off groups will taste current and prototype on day 7 only and visually assess a gray tented sample.</b>
# of Completes:	CLT: n=100 per product :45 min Peel-offs: n=15 (5 respondents in 4 groups)
Study Location:	Minneapolis, MN

#### KEY DATES

Project Approval:	10/3/14
Samples Due at Site:	10/22 (Client will deliver product by <u>10:00am</u> the same day of the test; 3-digit codes generated by FPI) First session begins at 12:30
Test Date:	10/27/14
Final Deliverable:	11/10/14



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**CONFIDENTIAL**

WIN-91PKE6392EJ3a92e143-914f-4897-ba10  
03e495a8973e09935- DE- Meat Discoloration CLT  
Cost Estimate R03.00



1

## STUDY OVERVIEW

Background:	Deli Express has developed Ham Single Wedged sandwiches in flexible pouch packaging with clear top film. Several key customers have inquired about the discoloration of meat in the sandwiches. The R&D team would like to understand <b>consumer's recognition of meat discoloration and attitudes toward scavengers.</b>
Business Objective:	To protect Deli Express share of <b>cured meat</b> sandwiches by addressing customers concerns about discoloration.
Research Objective:	<ol style="list-style-type: none"> <li>1. To determine which sample is preferred between the current and prototype pairs <b>and degree of preference.</b></li> <li>2. To determine if new packaging solution will meaningfully increase purchase interest and liking of Ham Single-Wedge Sandwiches.</li> <li>3. To understand consumer's likes/dislikes about antioxidant scavengers.</li> </ol>
Action Standard:	Purchase Interest, Preference, and Visual Liking $\geq$ current

## RESEARCH DESIGN OVERVIEW

Study Type:	Visual Central Location Test (CLT) + Peel-offs (Blind)
Products Tested:	Ham Single-Wedged Sandwiches <ul style="list-style-type: none"> <li>• Current Day <b>4</b> + Prototype Day <b>4</b></li> <li>• Current Day <b>7</b> + Prototype Day <b>7</b></li> <li>• Current Day 30 + Prototype Day 30</li> </ul> <i>*All CLT sandwiches will be bunched*</i>
Products per Respondent:	All respondents will see all 3 pairs, paired comparison, fully rotated and balanced
Peel-off Group Orientation:	Four groups of 5 respondents- mixture of respondents that chose current and prototype with scavenger. <b>The peel-off groups will taste current and prototype on day 7 only and visually assess a gray tented sample.</b>
# of Completes:	CLT: n=100 per product :45 min Peel-offs: n=15 (5 respondents in 4 groups)
Study Location:	Minneapolis, MN
Product Quantities Needed:	Currently targeting 100 of each variable

**SCREENING CRITERIA**

FPI Standard:	Security Screen, No food allergies, No dietary restrictions, <b>Not color blind</b>
Past Participation:	No participation in the past 60 days for quantitative
Food Responsibility:	N/A
Age:	18-54 years old
Gender:	50:50 male:female
Kids/No Kids:	N/A
HH Income:	As it falls
Ethnicity:	As it falls
Category Usage:	Have purchased fresh, pre-packaged sandwiches from a convenient store such as breakfast sandwiches, hamburgers, deli meat sandwiches in the past 3 months
Brand Usage:	N/A
Concept Acceptors:	N/A – due to this being a blind study
Flavor Acceptors:	Like and willing to try ham and cured meat deli sandwiches
Articulation:	Respondents will be peeled-off based on the ability to stay and self-articulation in screener.
Estimated Incidence:	25%

**DATA COLLECTIONS & ANALYSIS**

# of close-ended questions:	9 per product (includes purchase intent, meets expectations, JAR, preference)
# of open-ended questions:	1 per product (10 per respondent)
Data Collection Method:	Paper
Analysis Approach:	ANOVA within and between pairs
Data Breaks to be Reported:	Total population
Open-end Delivery:	Verbatims



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WIN-91PKE6392EJ3a92e143-914f-4897-ba10  
 03e495a8973e09935- DE- Meat Discoloration CLT  
 Cost Estimate R03.00




3

## FPI DELIVERABLES

	<ul style="list-style-type: none"><li>• Research Design Consultation</li></ul>
	<ul style="list-style-type: none"><li>• Respondent recruitment</li></ul>
	<ul style="list-style-type: none"><li>• Material development including screener and questionnaire.</li></ul>
	<ul style="list-style-type: none"><li>• Test set-up, coordination, and administration, staffing and facility rental, including product preparation and handling</li></ul>
	<ul style="list-style-type: none"><li>• Data analysis and reporting</li></ul>
	<ul style="list-style-type: none"><li>• Provide lab area for colorimeter measurements</li></ul>
	<ul style="list-style-type: none"><li>• Generate 3-digit random codes for products</li></ul>
	<ul style="list-style-type: none"><li>• Dark refrigerated storage</li></ul>

## CLIENT DELIVERABLES

	<ul style="list-style-type: none"><li>• All products and preparation instructions</li></ul>
	<ul style="list-style-type: none"><li>• Product to arrive at least 3 business days before test date</li></ul>
	<ul style="list-style-type: none"><li>• Facilitator for peel-off interviews</li></ul>
	<ul style="list-style-type: none"><li>• Approval of project bid, screener and questionnaire content and other items, as requested to execute test</li></ul>
	<ul style="list-style-type: none"><li>• Microbiological clearance</li></ul>
	<ul style="list-style-type: none"><li>• Client will stage product and transport to FPI the day of the test</li></ul>
	<ul style="list-style-type: none"><li>• Client will code the products with 3-digit codes</li></ul>
	<ul style="list-style-type: none"><li>• Client will measure oxygen levels before consumers taste in peel-offs</li></ul>



## ADDENDUM A

---

The following terms and conditions apply to proposed project between Client and FPI (Food Perspectives Inc.)

### Facility Reservation & Guarantee Policy

FPI will reserve a facility with the appropriate size and amenities at the proper time and place to accommodate Client's testing requirements. In order to help FPI and its subcontractors manage their facilities, the following guidelines apply to Client:

1. Tentative Hold – Facility space will be held on a first come, first served basis. The facility will be held, free of charge, until another interested party contacts FPI or one of its subcontractor test sites or once Client has approved the project, whichever comes first.
2. Right of First Refusal – When another interest party contacts FPI or one of its subcontractor test sites, Client will be contacted.
  - a. Client can choose, free of charge, to move their test date to another open day and tentatively hold that new date; or,
  - b. Client can choose to guarantee specific test dates. Once guaranteed, if the test is cancelled or postponed or if the test date needs to be rescheduled, Client will be charged for full site fees (costs vary by location and testing needs).

NOTE: These guidelines apply to any test that has not been approved by Client (specifically meaning Client has not, in written or verbal form, committed to running the test). If a project has been approved by Client, then the Postponement & Cancellation Policy applies and not this Facility Reservation & Guarantee Policy.

### Postponement & Cancellation Policy

FPI will attempt to work with its clients to minimize the charges for postponed projects, as often the original test materials can be reused and other charges mitigated.

FPI maintains a cancellation schedule which is determined by the amount of time and money that FPI has invested in the project at the point that it is cancelled. It is structured to allow Client to maintain high levels of project flexibility at the lowest cost possible.

1. For tests cancelled\* after the test has been approved but prior to starting recruiting, Client will be charged for the following:
  - o Test design, set up and material development completed to date
  - o Site fees
  - o Subcontractor services (e.g. moderators, etc.), if applicable
  - o Other charges may also apply depending upon the nature of the test
- \* Cancellation charges dependent upon city (cities) the test is conducted in.
2. For tests cancelled after recruiting has begun, Client will be charged for items listed in #1 plus the following:
  - o Charges for recruiting, to date
  - o Charges to cancel respondents that have already been scheduled (or in the case of test postponement, charges to reschedule respondents who have already qualified)
  - o Respondent incentives for those who cannot be contacted prior the cancelled test
  - o Partial site labor, if employees have been scheduled
  - o Other charges may also apply depending upon the nature of the test
3. For test cancelled the week of the test, Client will be charged for items listed in #1 and #2 plus the following:
  - o Full site labor
  - o Other charges may also apply depending upon the nature of the test

#### Confidentiality

FPI maintains strict confidentiality with its clients and will not disclose any information received from and/or about Client Company or project results without written authorization from Client Company.

#### Consent of Use of FPI Name or Logos

The trademarks, service marks and logos ("FPI Trademarks") used and displayed in FPI's work product are registered or unregistered Trademarks of Food Perspectives, Inc and FPI Holdings, Inc. No part of our testing, including any work product delivered to the client, shall be construed as granting, by implication, estoppel or otherwise any license or right to use any FPI Trademarks displayed in the work product without the prior written consent of FPI's CEO and President. Furthermore, the names FPI, Food Perspectives, Inc., FPI Holdings or any Trademarks may not be used in any way (including, but not limited to, use in advertising, product claims or other distribution of materials) by Client Company without the prior written consent of FPI's CEO and President.

#### Credit Terms

FPI requires its invoices paid within 30 days from the invoice date. Accounts not paid within 30 days are subject to a 2% monthly finance charge.

#### Intellectual Property

FPI is the owner, licensee or sublicensee of various pre-existing methods, protocols, procedures, software, programs, models and tools, routines and/or other programs, techniques, databases and materials that FPI may use or implement in the performance of this project ("FPI Materials"). FPI retains all right, title and interest in and to the FPI Materials. Client is not authorized to sell or license any FPI Materials or rights thereto to any other person or firm. FPI shall retain all right, title, and interest in any intellectual property created or developed by FPI that modifies or improves its FPI Materials as a result of completing this project.

#### Limited Liability

In the final deliverable to Client Company, FPI warrants only that its conclusions regarding the project are statistically significant, or insignificant, whatever the case may be. FPI is not warranting that any decision or course of action Client Company takes based on the final deliverable will be successful or, as the case may be, unsuccessful. In no event will FPI be liable to customer for any lost profits, lost savings or incidental, indirect, special or consequential damages arising out of Client Company's course of action based on FPI's final deliverable.

#### Multi-city tests

Airline tickets will be purchased approximately 14 days in advance if possible at costs available at the time. Tickets are generally non-refundable.

- All costs incurred by alternate sites will be passed to client if tests are cancelled less than 2 weeks prior to test date.

#### Publicity

Neither Client Company nor FPI will disclose their relationship, the contents of this estimate, or any information regarding the project to a third party without prior written approval.

#### Respondent Recruiting

- Approved respondent screening criteria are due from our client one business day prior to starting recruiting. Recruiting typically starts two weeks prior to the date that the test is scheduled. (Occasionally, FPI may ask its client to approve screening criteria early due to a holiday, low incidence, restrictive screening criteria, etc.)
- Respondents will always be slightly over recruited to get desired n. Over recruits will be included in tests if product quantities are sufficient or paid and sent away.
- Food Perspectives reserves the right to re-bid a project if recruiting incidence varies from proposal or recruiting criteria changes.

"\* To the extent that FPI has a Master Service Agreement with Client Company, where these terms conflict with any Master Service Agreement terms, the Master Service Agreement prevails. \*\*



## J.17 Respondent acknowledgement form for consumer test

07554

Resp. \_\_\_\_\_

### RESPONDENT ACKNOWLEDGEMENT

Thank you for participating in Food Perspectives, Inc.'s ("FPI") consumer research study.

This research study is being conducted to obtain consumer input about various products. Upon participation in our tests, your opinion, comments, responses and/or results will be shared with our research sponsor. Before participating in the research study, please read and acknowledge the following:

#### **VOLUNTARY**

Participation in this research study is completely voluntary and done at your own risk. You agree that you personally assume all risks for any loss, damage or injury to you or others, arising from, or in connection with, this research study and that you release both FPI and the research sponsor from any liability for any such loss, damage or injury.

#### **CONFIDENTIAL**

Because the products you will see today are not yet publicly available, we ask that you keep the products and any other information confidential. By signing this acknowledgement, you acknowledge your agreement to keep the products and associated information you learn as part of this research study completely confidential. That means that information from this research study must not be shared with your family, friends or any other individual and that no product or portion thereof may be removed from the research study area, unless you are instructed to do so. Additionally, your agreement to keep these products and related information confidential is indefinite or lasts until the products tested have been made publicly available.

#### **ALLERGIES & SENSITIVITIES**

For this research study, we will ask you to sample one or more food products. For your comfort and safety, we want to ensure that you do not test any products to which you might be allergic. (Please check the appropriate box below.)

☐ I **DO NOT** have any known conditions, allergies, or sensitivities that could prevent me from completing this research study safely.

☐ I **DO** have a condition, allergy, or sensitivity that could prevent me from completing this research study safely.

#### **OWNERSHIP**

The statements you will make will reflect your actual, authentic personal opinions, experiences and feedback. You acknowledge that you will not be prompted by employees, agents or representatives of the research sponsor to make any rehearsed statements. If you are prompted by anyone, please notify a research staff member immediately, unless you are simply asked to repeat your own thoughts, reactions, feedback and/or ideas.

If in your responses as a participant in this research project you provide any ideas, comments or suggestions about the products and related information you are shown, you agree that such responses will become the sole property of the research sponsor upon submission and that the research sponsor is not required to keep such responses confidential and is free to use them for any purpose whatsoever without additional payment or compensation to you.



By signing this statement, you understand that the research sessions you will be taking part in today could be photographed, videotaped, and/or audio taped. You hereby allow the research sponsor, to use, reproduce, and/or publish photographs and/or video that may pertain to you – including your name, image, likeness and/or voice – in any manner the research sponsor deems appropriate in order to promote or publicize its services and/or products. You understand that this material may be used in various publications, public affairs releases, recruitment materials, web pages and/or websites, broadcast public service advertising (PSAs) or for other related endeavors.

This authorization is continuous and may only be withdrawn by your specific written rescission of this authorization.

Nothing herein will constitute any obligation on the research sponsor to exercise any of the above rights.

#### **PAYMENT**

You represent and warrant that you have reached the age of majority and/or that you are the legal parent or guardian of a minor respondent and therefore can grant the rights hereunder. You acknowledge that the rights granted hereunder will not conflict with or violate any commitment, agreement, or understanding you and/or the minor respondent(s) have with any other person or entity.

Furthermore, you hereby acknowledge that you and/or the minor respondent(s) will be receiving payment for participating in this research. You understand that no additional payment is to be made for the use of any of your responses such as survey answers, interviews, photos, video or audio taping or other research activities.

Your cooperation and support are very much appreciated. Thank you!

**I acknowledge that I have read the statement above carefully. My signature below confirms that I have read and understood the contents of this document.**

By:

\_\_\_\_\_  
Print Name

\_\_\_\_\_  
Signature

Date: \_\_\_\_\_

On behalf of:

Minor child name(s) participating in the research study (list all):

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



J.18 Raw data from consumer test

A	B	C	D	E
	Product Code			
	Current Day 4 -		Prototype Day 4 -	
	Mean	Standard Deviation	Mean	Standard Deviation
Overall Liking of Appearance	5.8	1.73	6.5	1.43
Liking of Meat Color	5.9	1.83	6.8	1.35

A	B	C	D	E
	Product Code			
	Current Day 7 -		Prototype Day 7 -	
	Mean	Standard Deviation	Mean	Standard Deviation
Overall Liking of Appearance	6.7	1.27	6.2	1.49
Liking of Meat Color	6.7	1.27	6.3	1.46

A	B	C	D	E
	Product Code			
	Current Day 30 -		Prototype Day 30 -	
	Mean	Standard Deviation	Mean	Standard Deviation
Overall Liking of Appearance	5.3	1.85	5.4	1.72
Liking of Meat Color	5.1	2.08	5.5	1.82

Resp ID	Record #	Prod Code	Look: O-all L/D Appear	Look: L/D Meat Color	Look: Rarte Meat Color	Look: How Likely Purchase	Look: Meet Expectations	Prod Code Prefer O-all	Gender	Age	Ethnic Background	Last Level School Completed	# People In HH	Total Income
1	1	397	4	7	3	4	3	397	1	2	6	1	3	4
1	2	840	4	6	3	4	3	397	1	2	6	1	3	4
1	3	168	4	6	4	3	3	168	1	2	6	1	3	4
1	4	559	4	8	3	4	4	168	1	2	6	1	3	4
1	5	783	4	2	1	2	2	783	1	2	6	1	3	4
1	6	426	4	4	2	2	2	783	1	2	6	1	3	4
2	1	840	7	8	3	4	3	397	1	5	6	5	2	6
2	2	397	7	8	3	4	3	397	1	5	6	5	2	6
2	3	559	9	8	3	4	3	559	1	5	6	5	2	6
2	4	168	7	7	3	4	3	559	1	5	6	5	2	6
2	5	426	8	8	3	4	3	426	1	5	6	5	2	6
2	6	783	6	4	3	3	2	426	1	5	6	5	2	6
3	1	168	7	7	3	4	4	168	2	3	6	5	2	3
3	2	559	7	6	3	4	4	168	2	3	6	5	2	3
3	3	783	5	4	2	3	3	426	2	3	6	5	2	3
3	4	426	6	6	3	3	3	426	2	3	6	5	2	3
3	5	397	7	7	3	4	4	397	2	3	6	5	2	3
3	6	840	7	7	3	4	4	397	2	3	6	5	2	3
4	1	559	7	7	3	3	3	559	2	4	6	5	3	6
4	2	168	7	7	3	3	3	559	2	4	6	5	3	6
4	3	426	4	4	2	2	2	783	2	4	6	5	3	6
4	4	783	6	5	2	2	2	783	2	4	6	5	3	6
4	5	840	6	4	4	3	3	397	2	4	6	5	3	6
4	6	397	7	8	3	4	4	397	2	4	6	5	3	6
5	1	783	5	8	3	2	3	783	1	5	6	4	3	4
5	2	426	4	7	3	2	3	783	1	5	6	4	3	4
5	3	397	5	8	3	2	3	397	1	5	6	4	3	4
5	4	840	4	5	2	2	3	397	1	5	6	4	3	4
5	5	168	6	6	2	2	3	559	1	5	6	4	3	4
5	6	559	6	5	2	2	3	559	1	5	6	4	3	4
6	1	426	6	7	3	4	3	426	1	4	3	3	3	1
6	2	783	5	7	3	3	4	426	1	4	3	3	3	1
6	3	840	7	6	3	4	4	840	1	4	3	3	3	1
6	4	397	5	7	4	3	3	840	1	4	3	3	3	1
6	5	559	8	9	3	4	3	559	1	4	3	3	3	1
6	6	168	6	5	5	4	5	559	1	4	3	3	3	1
7	1	397	8	9	3	4	4	397	2	4	6	4	3	3
7	2	840	8	7	4	4	4	397	2	4	6	4	3	3
7	3	783	6	3	2	2	3	783	2	4	6	4	3	3
7	4	426	2	1	5	1	1	783	2	4	6	4	3	3
7	5	168	8	7	4	4	4	168	2	4	6	4	3	3
7	6	559	4	3	3	2	2	168	2	4	6	4	3	3
8	1	840	7	7	3	4	3	397	2	3	6	5	2	5
8	2	397	8	7	3	4	4	397	2	3	6	5	2	5
8	3	426	3	4	4	2	2	783	2	3	6	5	2	5
8	4	783	4	4	2	2	2	783	2	3	6	5	2	5
8	5	559	5	5	3	3	3	168	2	3	6	5	2	5
8	6	168	6	6	3	4	3	168	2	3	6	5	2	5
9	1	168	8	7	2	3	3	168	2	4	6	3	1	2
9	2	559	4	4	4	2	2	168	2	4	6	3	1	2
9	3	397	7	7	2	3	3	840	2	4	6	3	1	2
9	4	840	8	8	3	4	4	840	2	4	6	3	1	2
9	5	783	8	8	3	4	4	783	2	4	6	3	1	2
9	6	426	4	4	4	2	2	783	2	4	6	3	1	2
10	1	559	6	6	3	4	4	168	1	5	3	5	3	4
10	2	168	7	7	3	4	4	168	1	5	3	5	3	4
10	3	840	7	5	3	4	3	397	1	5	3	5	3	4
10	4	397	8	8	3	5	4	397	1	5	3	5	3	4
10	5	426	5	4	4	3	2	426	1	5	3	5	3	4
10	6	783	5	5	4	3	2	426	1	5	3	5	3	4
12	1	426	4	7	3	3	2	783	2	3	6	5	3	4
12	2	783	5	4	2	3	3	783	2	3	6	5	3	4
12	3	559	6	6	3	3	3	168	2	3	6	5	3	4
12	4	168	7	7	3	4	4	168	2	3	6	5	3	4
12	5	840	6	6	3	3	4	840	2	3	6	5	3	4
12	6	397	2	2	1	1	2	840	2	3	6	5	3	4
13	1	397	8	7	3	4	4	840	1	3	6	2	2	7
13	2	840	8	8	3	5	5	840	1	3	6	2	2	7
13	3	559	5	5	2	3	3	168	1	3	6	2	2	7
13	4	168	7	6	4	4	4	168	1	3	6	2	2	7
13	5	426	5	4	4	2	2	426	1	3	6	2	2	7
13	6	783	1	1	5	1	1	426	1	3	6	2	2	7
14	1	840	6	7	3	4	3	840	1	4	6	4	3	5
14	2	397	6	6	3	3	3	840	1	4	6	4	3	5
14	3	168	4	4	4	2	2	559	1	4	6	4	3	5
14	4	559	5	6	3	4	3	559	1	4	6	4	3	5
14	5	783	4	4	4	2	2	426	1	4	6	4	3	5
14	6	426	4	4	4	2	2	426	1	4	6	4	3	5
15	1	168	6	7	3	2	3	168	2	5	6	4	3	4
15	2	559	6	6	3	2	3	168	2	5	6	4	3	4
15	3	426	6	4	3	3	3	783	2	5	6	4	3	4
15	4	783	7	7	3	4	4	783	2	5	6	4	3	4
15	5	840	8	7	3	4	4	397	2	5	6	4	3	4
15	6	397	7	4	2	3	3	397	2	5	6	4	3	4
16	1	559	7	7	3	4	4	168	1	5	3	5	2	3
16	2	168	8	8	3	4	4	168	1	5	3	5	2	3
16	3	783	8	8	3	5	4	426	1	5	3	5	2	3
16	4	426	8	8	3	5	4	426	1	5	3	5	2	3
16	5	397	8	8	3	5	4	840	1	5	3	5	2	3
16	6	840	8	8	3	5	4	840	1	5	3	5	2	3
11	1	783	3	3	2	1	2	426	2	2	6	2	2	1
11	2	426	4	6	3	2	3	426	2	2	6	2	2	1
11	3	168	3	5	3	2	2	559	2	2	6	2	2	1
11	4	559	6	6	3	3	4	559	2	2	6	2	2	1
11	5	397	2	2	1	1	1	840	2	2	6	2	2	1
11	6	840	4	4	2	2	2	840	2	2	6	2	2	1
17	1	783	2	4	2	1	2	426	2	5	6	7	3	6
17	2	426	3	7	3	3	3	426	2	5	6	7	3	6
17	3	840	7	7	3	4	4	840	2	5	6	7	3	6



17	4	397	4	4	2	3	2	840	2	5	6	7	3	6
17	5	559	6	7	3	3	3	168	2	5	6	7	3	6
17	6	168	7	6	3	4	3	168	2	5	6	7	3	6
18	1	426	7	8	3	4	4	426	2	4	6	2	2	2
18	2	783	4	5	4	2	2	426	2	4	6	2	2	2
18	3	397	6	5	4	3	2	840	2	4	6	2	2	2
18	4	840	8	7	3	4	5	840	2	4	6	2	2	2
18	5	168	6	7	3	4	4	559	2	4	6	2	2	2
18	6	559	8	7	3	4	4	559	2	4	6	2	2	2
19	1	397	7	7	3	4	3	840	1	4	6	3	2	3
19	2	840	8	8	3	5	4	840	1	4	6	3	2	3
19	3	426	6	6	3	2	2	426	1	4	6	3	2	3
19	4	783	4	3	1	1	2	426	1	4	6	3	2	3
19	5	559	7	6	3	4	3	168	1	4	6	3	2	3
19	6	168	8	8	3	5	5	168	1	4	6	3	2	3
20	1	840	7	7	3	4	4	840	1	3		6	1	6
20	2	397	5	5	2	3	3	840	1	3		6	1	6
20	3	783	4	3	1	2	2	426	1	3		6	1	6
20	4	426	5	6	4	3	3	426	1	3		6	1	6
20	5	168	6	6	3	3	4	559	1	3		6	1	6
20	6	559	6	6	3	3	4	559	1	3		6	1	6
21	1	168	7	7	4	3	3	168	2	4	6	5	3	5
21	2	559	4	6	3	3	3	168	2	4	6	5	3	5
21	3	840	7	6	3	4	4	840	2	4	6	5	3	5
21	4	397	4	4	2	2	2	840	2	4	6	5	3	5
21	5	426	7	7	3	4	3	426	2	4	6	5	3	5
21	6	783	5	4	4	2	2	426	2	4	6	5	3	5
22	1	559	6	6	3	4	4	168	2	4	6	5	4	4
22	2	168	6	7	3	4	4	168	2	4	6	5	4	4
22	3	397	5	4	2	3	3	840	2	4	6	5	4	4
22	4	840	6	6	3	4	3	840	2	4	6	5	4	4
22	5	783	5	4	2	3	2	426	2	4	6	5	4	4
22	6	426	7	6	3	4	4	426	2	4	6	5	4	4
23	1	783	5	7	3	3	3	783	1	5	6	6	1	2
23	2	426	6	4	4	2	3	783	1	5	6	6	1	2
23	3	559	6	7	3	4	3	559	1	5	6	6	1	2
23	4	168	6	7	3	3	3	559	1	5	6	6	1	2
23	5	840	5	6	3	3	3	840	1	5	6	6	1	2
23	6	397	5	4	2	2	3	840	1	5	6	6	1	2
24	1	426	6	8	3	3	3	426	1	5	6	5	2	7
24	2	783	6	6	2	2	2	426	1	5	6	5	2	7
24	3	168	8	8	3	4	4	559	1	5	6	5	2	7
24	4	559	8	8	3	4	4	559	1	5	6	5	2	7
24	5	397	7	7	3	3	3	840	1	5	6	5	2	7
24	6	840	8	8	3	4	3	840	1	5	6	5	2	7
25	1	397	8	6	3	5	5	397	2	2	6	2	2	4
25	2	840	4	3	4	2	3	397	2	2	6	2	2	4
25	3	168	7	6	2	3	2	168	2	2	6	2	2	4
25	4	559	3	3	5	1	1	168	2	2	6	2	2	4
25	5	426	4	2	5	2	2	783	2	2	6	2	2	4
25	6	783	8	6	2	4	4	783	2	2	6	2	2	4
26	1	840	8	8	3	5	3	840	1	5	6	6	3	6
26	2	397	7	6	2	4	3	840	1	5	6	6	3	6
26	3	559	4	4	4	3	3	559	1	5	6	6	3	6
26	4	168	4	6	3	4	3	559	1	5	6	6	3	6
26	5	783	4	6	3	3	2	783	1	5	6	6	3	6
26	6	426	4	4	4	3	2	783	1	5	6	6	3	6
27	1	168	4	7	3	2	2	559	1	5	6	5	2	5
27	2	559	7	7	3	3	3	559	1	5	6	5	2	5
27	3	783	5	4	2	2	2	426	1	5	6	5	2	5
27	4	426	5	6	3	3	3	426	1	5	6	5	2	5
27	5	840	5	5	2	3	2	840	1	5	6	5	2	5
27	6	397	4	4	2	2	2	840	1	5	6	5	2	5
28	1	559	8	8	3	4	4	559	2	5	5	3	2	2
28	2	168	8	8	3	4	4	559	2	5	5	3	2	2
28	3	426	7	7	2	3	3	783	2	5	5	3	2	2
28	4	783	8	8	3	5	4	783	2	5	5	3	2	2
28	5	397	8	8	3	4	4	397	2	5	5	3	2	2
28	6	840	7	8	3	3	3	397	2	5	5	3	2	2
29	1	783	7	8	3	3	3	783	2	3	6	3	4	7
29	2	426	4	3	4	2	2	783	2	3	6	3	4	7
29	3	840	8	8	3	4	4	840	2	3	6	3	4	7
29	4	397	2	2	5	1	1	840	2	3	6	3	4	7
29	5	168	6	6	3	2	3	168	2	3	6	3	4	7
29	6	559	3	2	4	2	2	168	2	3	6	3	4	7
30	1	426	5	6	2	3	2	783	1	5	6	7	2	6
30	2	783	7	4	1	4	4	783	1	5	6	7	2	6
30	3	397	6	7	3	4	4	840	1	5	6	7	2	6
30	4	840	6	8	3	4	4	840	1	5	6	7	2	6
30	5	559	7	7	3	4	4	168	1	5	6	7	2	6
30	6	168	8	8	3	4	4	168	1	5	6	7	2	6
31	1	397	9	9	3	5	5	397	2	5	6	4	2	6
31	2	840	8	8	4	3	3	397	2	5	6	4	2	6
31	3	783	7	8	3	3	3	783	2	5	6	4	2	6
31	4	426	4	3	3	1	1	783	2	5	6	4	2	6
31	5	559	8	7	2	4	3	168	2	5	6	4	2	6
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32	1	840	6	8	3	4	4	840	2	5	6	4	2	6
32	2	397	6	6	2	3	3	840	2	5	6	4	2	6
32	3	426	6	6	4	3	3	783	2	5	6	4	2	6
32	4	783	7	7	3	4	4	783	2	5	6	4	2	6
32	5	168	7	7	3	4	4	559	2	5	6	4	2	6
32	6	559	8	7	3	4	4	559	2	5	6	4	2	6
33	1	168	7	6	2	3	3	168	2	4	6	3	3	3
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33	3	397	4	4	2	2	2	397	2	4	6	3	3	3
33	4	840	5	6	3	2	2	397	2	4	6	3	3	3
33	5	426	4	4	4	2	2	783	2	4	6	3	3	3
33	6	783	6	6	3	4	3	783	2	4	6	3	3	3
34	1	559	7	7	3	4	3	168	1	5	3	4	1	3

34	2	168	8	8	3	5	4	168	1	5	3	4	1	3
34	3	840	7	7	4	3	2	397	1	5	3	4	1	3
34	4	397	7	7	2	4	4	397	1	5	3	4	1	3
34	5	783	4	3	4	2	2	426	1	5	3	4	1	3
34	6	426	7	7	2	4	4	426	1	5	3	4	1	3
35	1	783	4	5	2	3	3	426	2	5	6	5	2	7
35	2	426	7	7	3	4	4	426	2	5	6	5	2	7
35	3	559	7	7	3	4	4	168	2	5	6	5	2	7
35	4	168	7	7	3	4	4	168	2	5	6	5	2	7
35	5	397	5	5	2	3	3	840	2	5	6	5	2	7
35	6	840	7	7	3	4	4	840	2	5	6	5	2	7
36	1	426	4	3	2	2	2	783	2	2	2	5	2	1
36	2	783	6	6	3	3	2	783	2	2	2	5	2	1
36	3	168	7	7	3	3	3	559	2	2	2	5	2	1
36	4	559	6	5	3	4	2	559	2	2	2	5	2	1
36	5	840	7	7	3	4	3	840	2	2	2	5	2	1
36	6	397	3	4	4	2	3	840	2	2	2	5	2	1
37	1	397	6	7	3	3	3	397	1	5	6	3	2	3
37	2	840	6	6	3	3	3	397	1	5	6	3	2	3
37	3	559	6	6	3	3	3	168	1	5	6	3	2	3
37	4	168	6	6	3	3	3	168	1	5	6	3	2	3
37	5	783	6	6	3	3	3	783	1	5	6	3	2	3
37	6	426	6	6	3	3	3	783	1	5	6	3	2	3
38	1	840	6	7	3	4	3	840	2	4	6	3	1	4
38	2	397	6	6	2	3	2	840	2	4	6	3	1	4
38	3	168	7	7	3	4	3	168	2	4	6	3	1	4
38	4	559	6	4	2	2	2	168	2	4	6	3	1	4
38	5	426	4	6	3	2	2	783	2	4	6	3	1	4
38	6	783	4	4	4	2	2	783	2	4	6	3	1	4
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82	4	397	5	7	2	2	2	840	2	4	6	4	1	7
82	5	783	7	7	2	4	3	783	2	4	6	4	1	7
82	6	426	4	6	2	2	2	783	2	4	6	4	1	7
83	1	783	8	9	2	4	4	426	1	3	6	6	2	4
83	2	426	8	9	3	4	3	426	1	3	6	6	2	4
83	3	559	6	4	2	3	2	168	1	3	6	6	2	4
83	4	168	6	5	2	3	3	168	1	3	6	6	2	4
83	5	397	3	3	1	2	2	840	1	3	6	6	2	4
83	6	840	8	8	3	4	4	840	1	3	6	6	2	4
84	1	426	4	6	4	3	3	426	1	5	6	2	3	3

84	2	783	4	6	4	3	3	426	1	5	6	2	3	3
84	3	168	7	7	3	4	3	168	1	5	6	2	3	3
84	4	559	6	7	3	4	3	168	1	5	6	2	3	3
84	5	840	7	7	3	4	3	840	1	5	6	2	3	3
84	6	397	5	4	2	3	3	840	1	5	6	2	3	3
85	1	397	7	8	3	4	4	840	1	4	6	5	2	7
85	2	840	8	8	3	4	5	840	1	4	6	5	2	7
85	3	559	8	8	3	5	5	559	1	4	6	5	2	7
85	4	168	8	4	1	3	2	559	1	4	6	5	2	7
85	5	783	3	1	1	1	1	426	1	4	6	5	2	7
85	6	426	6	6	2	3	3	426	1	4	6	5	2	7
86	1	840	8	7	3	4	3	397	1	3	6	7	1	3
86	2	397	8	9	3	5	4	397	1	3	6	7	1	3
86	3	168	8	8	3	4	4	559	1	3	6	7	1	3
86	4	559	8	8	3	5	4	559	1	3	6	7	1	3
86	5	426	7	6	3	3	3	426	1	3	6	7	1	3
86	6	783	7	6	4	3	3	426	1	3	6	7	1	3
87	1	168	6	7	3	3	4	168	2	3	6	7	2	5
87	2	559	5	4	2	3	3	168	2	3	6	7	2	5
87	3	426	3	3	5	2	2	426	2	3	6	7	2	5
87	4	783	2	2	1	2	2	426	2	3	6	7	2	5
87	5	397	7	8	3	4	5	397	2	3	6	7	2	5
87	6	840	4	5	3	4	4	397	2	3	6	7	2	5
88	1	559	7	8	3	5	5	168	2	4	7	3	4	2
88	2	168	8	8	3	5	5	168	2	4	7	3	4	2
88	3	783	6	2	5	2	2	426	2	4	7	3	4	2
88	4	426	8	8	3	4	4	426	2	4	7	3	4	2
88	5	840	9	9	3	5	5	840	2	4	7	3	4	2
88	6	397	6	6	1	3	3	840	2	4	7	3	4	2
89	1	783	3	7	2	3	2	426	1	4	6	7	2	5
89	2	426	8	9	3	5	5	426	1	4	6	7	2	5
89	3	397	7	7	2	4	3	397	1	4	6	7	2	5
89	4	840	8	8	2	4	4	397	1	4	6	7	2	5
89	5	559	6	6	2	2	2	168	1	4	6	7	2	5
89	6	168	8	8	3	5	5	168	1	4	6	7	2	5
90	1	426	7	7	2	4	3	783	2	3	6	7	2	4
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90	4	397	7	7	2	4	3	840	2	3	6	7	2	4
90	5	168	7	7	3	4	4	168	2	3	6	7	2	4
90	6	559	7	7	4	3	3	168	2	3	6	7	2	4
91	1	397	8	7	3	4	5	397	1	4	6	2	2	2
91	2	840	4	5	3	3	2	397	1	4	6	2	2	2
91	3	426	6	7	3	4	4	426	1	4	6	2	2	2
91	4	783	8	8	3	5	4	426	1	4	6	2	2	2
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91	6	559	7	6	4	4	4	168	1	4	6	2	2	2
92	1	840	8	8	3	4	4	840	1	5	6	5	2	6
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92	3	783	7	7	2	4	3	426	1	5	6	5	2	6
92	4	426	8	8	3	5	4	426	1	5	6	5	2	6
92	5	559	8	8	3	4	4	559	1	5	6	5	2	6
92	6	168	8	7	2	4	3	559	1	5	6	5	2	6
93	1	168	7	8	3	4	3	559	1	5	6	5	2	5
93	2	559	7	7	3	4	3	559	1	5	6	5	2	5
93	3	840	5	6	2	3	3	397	1	5	6	5	2	5
93	4	397	7	6	2	3	3	397	1	5	6	5	2	5
93	5	783	6	6	3	3	3	426	1	5	6	5	2	5
93	6	426	7	7	3	4	3	426	1	5	6	5	2	5
94	1	559	4	6	3	3	3	559	2	4	6	4	1	2
94	2	168	4	4	2	3	3	559	2	4	6	4	1	2
94	3	397	4	3	1	2	3	840	2	4	6	4	1	2
94	4	840	5	6	3	3	3	840	2	4	6	4	1	2
94	5	426	3	3	2	2	2	783	2	4	6	4	1	2
94	6	783	3	3	1	2	2	783	2	4	6	4	1	2
95	1	783	9	8	3	5	3	783	2	3	2	1	2	2
95	2	426	9	6	4	5	3	783	2	3	2	1	2	2
95	3	168	5	7	3	5	3	168	2	3	2	1	2	2
95	4	559	4	5	3	4	2	168	2	3	2	1	2	2
95	5	840	2	4	3	2	1	397	2	3	2	1	2	2
95	6	397	6	5	3	4	3	397	2	3	2	1	2	2
96	1	426	4	7	3	2	3	783	2	5	6	5	3	5
96	2	783	5	4	2	3	4	783	2	5	6	5	3	5
96	3	559	6	7	3	3	4	559	2	5	6	5	3	5
96	4	168	4	4	2	2	2	559	2	5	6	5	3	5
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96	6	840	6	7	3	3	4	840	2	5	6	5	3	5
97	1	397	5	5	4	3	2	840	1	3	2	4	1	7
97	2	840	7	7	3	4	4	840	1	3	2	4	1	7
97	3	168	5	5	2	3	2	168	1	3	2	4	1	7
97	4	559	3	3	5	2	1	168	1	3	2	4	1	7
97	5	783	2	2	1	1	1	783	1	3	2	4	1	7
97	6	426	1	1	5	1	1	783	1	3	2	4	1	7
98	1	840	8	8	3	4	5	840	2	2	6	4	3	6
98	2	397	6	8	3	3	3	840	2	2	6	4	3	6
98	3	559	4	8	3	2	3	168	2	2	6	4	3	6
98	4	168	6	8	3	3	3	168	2	2	6	4	3	6
98	5	426	7	8	3	3	4	426	2	2	6	4	3	6
98	6	783	6	5	2	3	3	426	2	2	6	4	3	6
99	1	168	8	8	3	4	4	559	2	5	3	5	2	3
99	2	559	9	8	3	4	4	559	2	5	3	5	2	3
99	3	783	7	6	4	3	2	783	2	5	3	5	2	3
99	4	426	4	4	4	2	2	783	2	5	3	5	2	3
99	5	397	8	7	3	4	3	397	2	5	3	5	2	3
99	6	840	4	4	4	2	2	397	2	5	3	5	2	3
100	1	559	6	7	2	3	3	168	1	4	2	3	5	6
100	2	168	8	7	3	4	4	168	1	4	2	3	5	6
100	3	426	4	6	2	3	2	783	1	4	2	3	5	6
100	4	783	6	6	3	4	3	783	1	4	2	3	5	6
100	5	840	7	6	3	4	3	397	1	4	2	3	5	6



100	6	397	6	5	2	4	3	397	1	4	2	3	5	6
101	1	783	8	8	3	4	4	783	1	2	6	4	4	5
101	2	426	6	5	5	2	3	783	1	2	6	4	4	5
101	3	397	6	6	4	3	3	397	1	2	6	4	4	5
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101	6	559	8	7	4	4	5	559	1	2	6	4	4	5
102	1	426	4	5	3	2	2	426	1	5	6	7	2	3
102	2	783	4	5	3	2	2	426	1	5	6	7	2	3
102	3	840	5	5	3	2	2	397	1	5	6	7	2	3
102	4	397	6	6	3	3	3	397	1	5	6	7	2	3
102	5	559	8	7	3	4	5	559	1	5	6	7	2	3
102	6	168	8	7	3	4	4	559	1	5	6	7	2	3
103	1	397	4	4	4	2	3	840	2	3	6	5	2	6
103	2	840	6	6	3	4	3	840	2	3	6	5	2	6
103	3	783	6	4	5	2	3	426	2	3	6	5	2	6
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103	5	168	7	7	3	5	4	168	2	3	6	5	2	6
103	6	559	6	6	3	4	3	168	2	3	6	5	2	6
104	1	840	6	6	3	4	3	397	2	4	6	4	1	3
104	2	397	7	7	3	4	4	397	2	4	6	4	1	3
104	3	426	6	6	4	4	3	426	2	4	6	4	1	3
104	4	783	3	3	2	2	2	426	2	4	6	4	1	3
104	5	559	6	6	3	4	3	559	2	4	6	4	1	3
104	6	168	5	5	2	3	2	559	2	4	6	4	1	3
105	1	168	8	8	3	5	4	559	1	5	6	4	1	4
105	2	559	8	8	3	5	4	559	1	5	6	4	1	4
105	3	397	8	8	3	5	3	840	1	5	6	4	1	4
105	4	840	9	8	3	5	5	840	1	5	6	4	1	4
105	5	783	6	5	2	3	2	426	1	5	6	4	1	4
105	6	426	8	8	2	4	3	426	1	5	6	4	1	4
106	1	559	6	6	3	3	3	559	1	3	2	5	3	4
106	2	168	5	6	3	3	2	559	1	3	2	5	3	4
106	3	840	5	5	3	3	3	397	1	3	2	5	3	4
106	4	397	6	6	3	3	3	397	1	3	2	5	3	4
106	5	426	6	5	4	3	2	783	1	3	2	5	3	4
106	6	783	6	6	3	3	3	783	1	3	2	5	3	4
107	1	783	6	6	4	3	3	426	2	5	6	4	2	3
107	2	426	5	6	3	3	3	426	2	5	6	4	2	3
107	3	168	8	8	3	4	4	168	2	5	6	4	2	3
107	4	559	6	4	2	3	3	168	2	5	6	4	2	3
107	5	397	4	5	2	2	2	840	2	5	6	4	2	3
107	6	840	6	7	3	4	3	840	2	5	6	4	2	3
108	1	426	7	6	5	4	3	783	2	5	6	6	2	7
108	2	783	7	7	3	4	4	783	2	5	6	6	2	7
108	3	559	7	7	3	4	4	168	2	5	6	6	2	7
108	4	168	7	8	3	5	4	168	2	5	6	6	2	7
108	5	840	3	3	1	2	2	840	2	5	6	6	2	7
108	6	397	3	3	1	1	2	840	2	5	6	6	2	7
109	1	397	7	8	3	4	4	840	1	3	6	6	2	2
109	2	840	7	8	3	4	4	840	1	3	6	6	2	2
109	3	559	7	7	2	4	4	168	1	3	6	6	2	2
109	4	168	7	6	3	4	4	168	1	3	6	6	2	2
109	5	426	7	5	2	3	3	426	1	3	6	6	2	2
109	6	783	4	4	2	2	2	426	1	3	6	6	2	2
110	1	840	7	8	4	3	3	840	1	2	3	2	2	2
110	2	397	2	4	2	1	2	840	1	2	3	2	2	2
110	3	168	6	8	3	3	2	559	1	2	3	2	2	2
110	4	559	5	6	2	4	3	559	1	2	3	2	2	2
110	5	783	2	1	4	1	1	426	1	2	3	2	2	2
110	6	426	3	4	4	2	2	426	1	2	3	2	2	2

## Appendix K Test 11 - Follow up tests after Test 10

### K.1 Summary of visual appearance of control and test sandwiches.

Date Collected	2/2/15	
MOCON	C1 A	S1A
Observations		
MOCON - CO <sub>2</sub> (%)	17.7	15.5
MOCON - O <sub>2</sub> (%)	0	0
Colorimeter	C1 A	S1A
L* (1) TOP	58.40	56.08
a* (1)	13.54	18.59
b* (1)	7.34	5.24
L* (2) MID	58.79	58.48
a* (2)	12.64	17.83
b* (2)	7.39	5.39
L* (3) END	59.51	59.48
a* (3)	10.81	17.53
b* (3)	8.12	6.35
L* AVERAGE	58.90	58.01
a* AVERAGE	12.33	17.98
b* AVERAGE	7.62	5.66
OVERALL AVERAGE L*		
OVERALL AVERAGE a*		
OVERALL AVERAGE b*		

-0.89

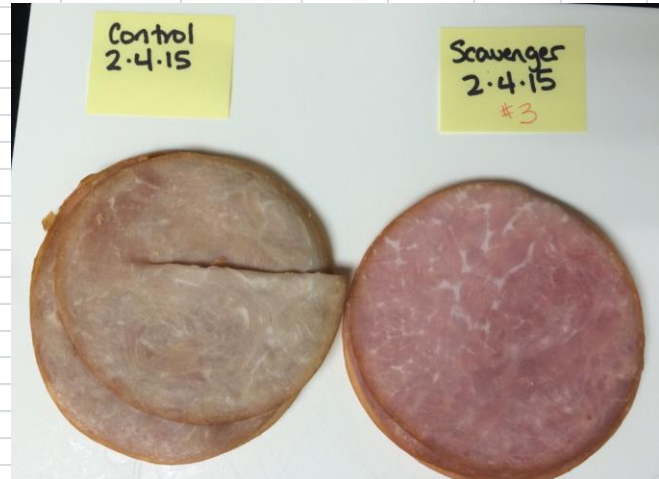
5.65

-1.96

Date Collected	2/3/15	
MOCON	C2 A	S2 A
Observations		
MOCON - CO <sub>2</sub> (%)	17.4	14.5
MOCON - O <sub>2</sub> (%)	0.00	0.00
Colorimeter	C2 A	S2 A
L* (1) TOP	52.02	56.69
a* (1)	9.70	13.66
b* (1)	5.67	6.48
L* (2) MID	51.81	57.49
a* (2)	8.92	13.32
b* (2)	5.99	6.11
L* (3) END	53.63	56.71
a* (3)	7.47	14.42
b* (3)	6.73	6.56
L* AVERAGE	52.49	56.96
a* AVERAGE	8.70	13.80
b* AVERAGE	6.13	6.38

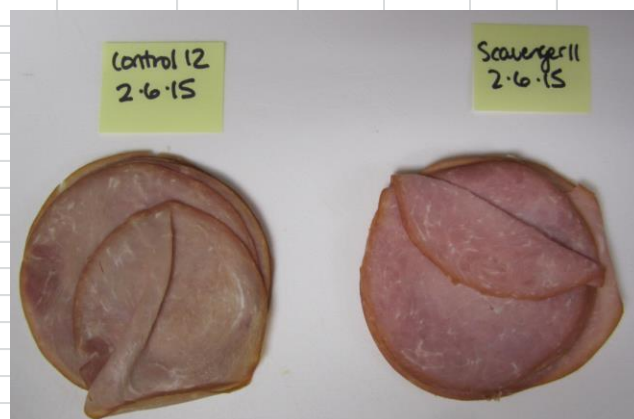
<b>Date Collected</b>	2/4/15	
<b>MOCON</b>	C3 A	S3 A
<b>Observations</b>		
MOCON - CO2 (%)	16.4	14.1
MOCON - O2 (%)	0.193	0
<b>Colorimeter</b>	C3 A	S3 A
L* (1) TOP	53.49	46.88
a* (1)	6.58	16.76
b* (1)	7.78	5.25
L* (2) MID	52.81	48.22
a* (2)	6.51	16.29
b* (2)	6.88	4.94
L* (3) END	52.43	52.14
a* (3)	7.29	15.66
b* (3)	7.51	5.04
<b>L* AVERAGE</b>	52.91	49.08
<b>a* AVERAGE</b>	6.79	16.24
<b>b* AVERAGE</b>	7.39	5.08



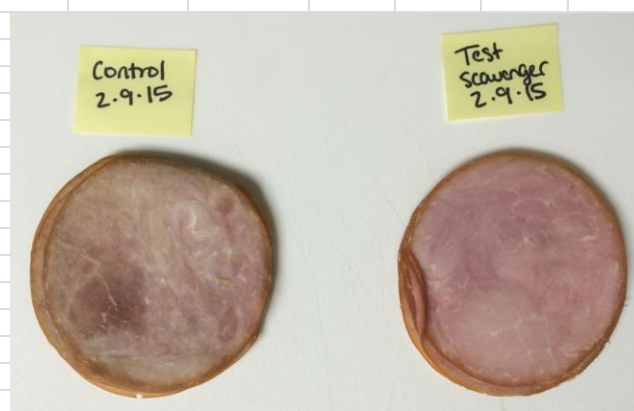
<b>Date Collected</b>	2/5/15	
<b>MOCON</b>	C4 A	S4 A
<b>Observations</b>		
MOCON - CO2 (%)	18	14.8
MOCON - O2 (%)	0.13	0
<b>Colorimeter</b>	C4 A	S4 A
L* (1) TOP	55.36	49.07
a* (1)	7.08	15.45
b* (1)	8.20	5.23
L* (2) MID	56.21	49.86
a* (2)	7.19	15.23
b* (2)	7.93	5.13
L* (3) END	55.25	49.98
a* (3)	8.14	15.39
b* (3)	7.94	5.25
<b>L* AVERAGE</b>	55.61	49.64
<b>a* AVERAGE</b>	7.47	15.36
<b>b* AVERAGE</b>	8.02	5.20



<b>Date Collected</b>	2/6/15	
<b>MOCON</b>	C1 A	S1 A
<i>Observations</i>		
MOCON - CO2 (%)	17.4	14.1
MOCON - O2 (%)	0	0
<b>Colorimeter</b>	C4 A	S4 A
L* (1) TOP	51.05	52.28
a* (1)	9.39	16.11
b* (1)	9.01	7.06
L* (2) MID	51.72	54.02
a* (2)	9.31	15.61
b* (2)	7.50	7.12
L* (3) END	51.80	54.29
a* (3)	9.01	15.19
b* (3)	8.20	6.55
<b>L* AVERAGE</b>	51.52	53.53
<b>a* AVERAGE</b>	9.24	15.64
<b>b* AVERAGE</b>	8.24	6.91



<b>Date Collected</b>	2/9/15	
<b>MOCON</b>	C1 A	S1 A
<i>Observations</i>		
MOCON - CO2 (%)	16.9	13.8
MOCON - O2 (%)	0	0
<b>Colorimeter</b>	C4 A	S4 A
L* (1) TOP	52.62	51.62
a* (1)	7.54	14.92
b* (1)	7.52	5.55
L* (2) MID	53.85	53.18
a* (2)	8.10	14.73
b* (2)	7.12	6.08
L* (3) END	51.51	56.26
a* (3)	9.15	14.03
b* (3)	6.51	6.38
<b>L* AVERAGE</b>	52.66	53.69
<b>a* AVERAGE</b>	8.26	14.56
<b>b* AVERAGE</b>	7.05	6.00



<b>Date Collected</b>	2/10/15	
<b>MOCON</b>	C1 A	S1 A
<i>Observations</i>		
MOCON - CO2 (%)	17	14.3
MOCON - O2 (%)	0.001	0
<b>Colorimeter</b>	C4 A	S4 A
L* (1) TOP	56.44	50.51
a* (1)	6.04	16.02
b* (1)	6.62	5.42
L* (2) MID	54.82	52.31
a* (2)	7.45	15.14
b* (2)	6.16	5.16
L* (3) END	52.35	52.82
a* (3)	8.74	14.55
b* (3)	6.04	4.81
L* AVERAGE	54.54	51.88
a* AVERAGE	7.41	15.24
b* AVERAGE	6.27	5.13

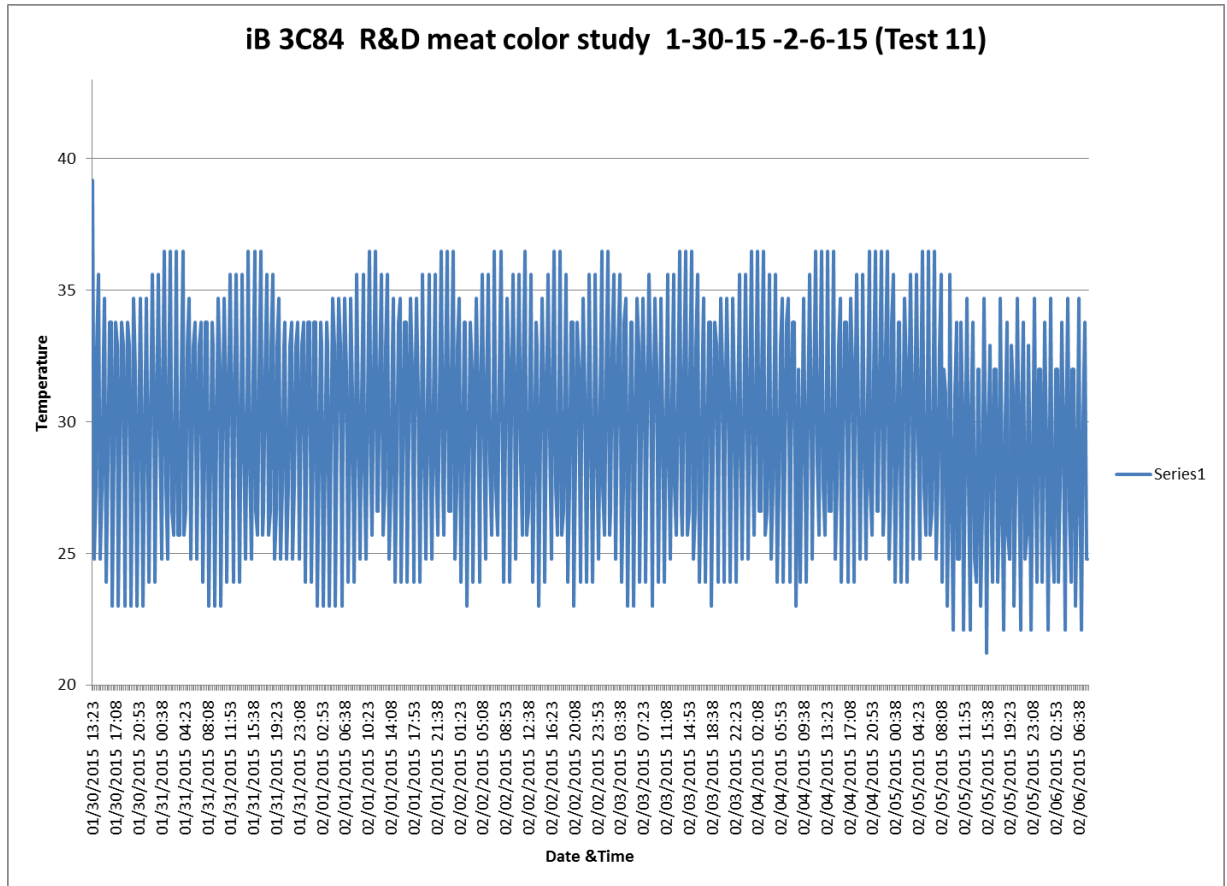


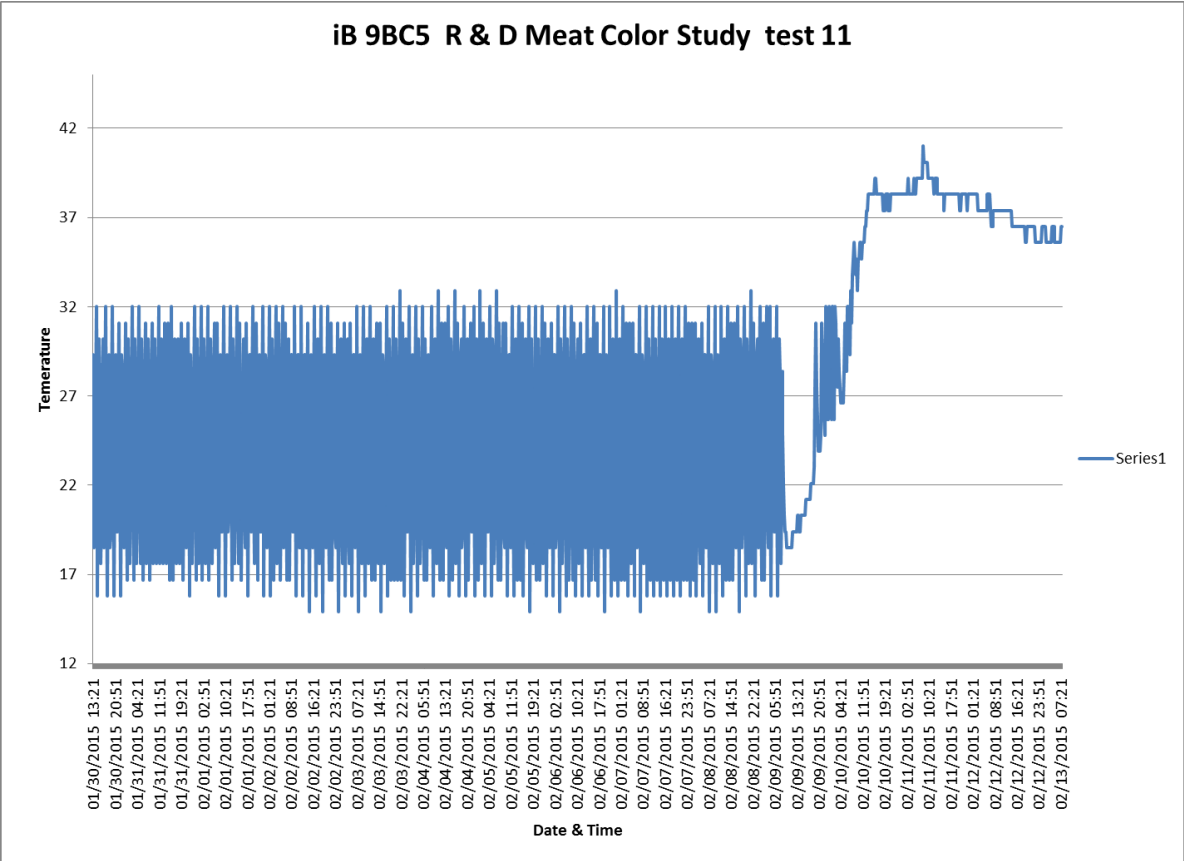
<b>Date Collected</b>	2/11/15	
<b>MOCON</b>	C1 A	S1 A
<i>Observations</i>		
MOCON - CO2 (%)	17.6	14.5
MOCON - O2 (%)	0	0
<b>Colorimeter</b>	C4 A	S4 A
L* (1) TOP	51.67	48.81
a* (1)	10.60	15.78
b* (1)	5.63	4.84
L* (2) MID	51.84	47.04
a* (2)	10.65	16.98
b* (2)	5.60	4.78
L* (3) END	51.74	45.39
a* (3)	10.64	18.20
b* (3)	5.65	5.10
L* AVERAGE	51.75	47.08
a* AVERAGE	10.63	16.99
b* AVERAGE	5.63	4.91



<b>Date Collected</b>	2/12/15	
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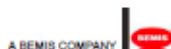
## K.2 Cooler temperature tracking Test 11 (Fahrenheit)





## Appendix L

### L.1 Curwood Technical Bulletin forming film 9581-AA



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## TECHNICAL BULLETIN

# CURLON® GRADE 9581-AA

## Protective Packaging Film

### APPLICATIONS

CURLON® (Grade 9581-AA) is a flexible, formable web for protective packaging of products which are suitable for vacuum and gas applications where low O<sub>2</sub> levels are required. Recommended for high speed packaging applications where package clarity, outside package C.O.F., uniform formed distribution, and package tightness are critical package criteria.

### FEATURES

Clear, Polyethylene sealant with excellent O<sub>2</sub> barrier properties and enhanced nylon for better thermoformability. Suitable for use in combination with CURLAM® non-forming webs.

### MACHINERY

Thermoformable HFFS, i.e. Die-less equipment

### FILM CONSTRUCTION

Proprietary Coextruded film with EVOH barrier, Polyethylene sealant and Nylon structural layers.

Nominal Thickness: 7.0 mil

FDA & USDA Status: CURLON® (Grade 9581-AA) meets the requirements for a food contact material under the Food Additive Regulations. Curwood has on file statements from suppliers of all materials used, assuring that the items we purchase are in compliance with the appropriate Food Additive Regulation.

### TYPICAL FILM PROPERTIES

Basis Wt.: 117.2 #/mm +/- 7.5%

Yield: 3,690 in<sup>2</sup>/lb

Seal strength: 3,000 gm/in minimum to a known polyethylene sealant.

#### Barrier Properties

O<sub>2</sub> < 0.30 CC per 100 in<sup>2</sup> per 24 Hrs @  
73°F & 0% RH

MVTR < 0.5 gm H<sub>2</sub>O per 100 in<sup>2</sup> per 24  
Hrs @ 100°F & 90% RH

3/30/07

#### IMPORTANT NOTICE TO PURCHASER

All statements, technical information, and recommendations contained herein are based on tests we believe to be reliable, but the accuracy or completeness thereof is not guaranteed, other sampling or test procedures may produce different values or results. Before using, user shall determine the suitability of the product or information for his intended use by appropriate testing, sampling and statistical analysis, and user assumes all risk and liability whatsoever in connection therewith. No statement or suggestion herein is to be considered a recommendation or inducement of any use, manufacture or sale that may infringe any patents now or hereafter in existence.